

2001116439

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RESEARCH CENTER  
RICHMOND, VIRGINIA

MONTHLY PROGRESS REPORTS

Period Covered

October 1 - 31, 1985

Date Issued

November 15, 1985

2001116440

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Product Development

**2001116443**

CHARGE NUMBER: 1990

PROJECT TITLE: BLEND DEVELOPMENT

PROJECT LEADER: C. M. MOOGALIAN

PERIOD COVERED: OCTOBER, 1985

I. Merit Select

Nine models are to be made incorporating the following DIET expansions:

1. Special Bright (Thin), 24 Mo. Duration
2. Special Bright (Thin), 12 Mo. Duration
3. Special Oriental
4. DBC Bright Control from same tobacco stocks as #1.

The models contain the following fillers:

1. Merit (Control)
2. Merit with Special Oriental Expanded
3. Merit with Special Bright Expanded (12 mos.)
4. Merit with Special Bright Expanded (24 mos.), With and Without Aftercut
5. Marlboro (Control)
6. Marlboro with Special Bright Expanded (12 mos.)
7. Marlboro with Special Bright Expanded (24 mos.), With and Without AC

The expansions have been completed, and models are expected to be made the first week in November.

Also, 100% cigarettes of special components used in this study (special DIET's and special strips) will be made.

II. PM Super Lights

In cooperation with the Leaf Department, the Super Lights blend has been formulated from U.S. tobacco inventories.

For this effort, a Special Bright expansion was run which utilized bodied tobaccos.

The cigarette model will be completed by the second week in November.

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### III. Dunhill Lights

The following tests are being conducted by Marketing Research:

Dunhill Lts. 85 vs. Win. Lts. 85  
HTI 5190 HTI 10098

Dunhill Lts. 100 vs. Win. Lts. 100  
HTI 5191 HTI 10099

Dunhill Lts. 85 vs. Marl. Lts. 85  
HTI 5192 HTI 1275

Dunhill Lts. 100 vs. Marl. Lts. 100  
HTI 5193 HTI 1276

The cigarettes have been made and shipped. Results are expected in late November.

#### IV. B & H Menthol With 3% Dark Air Cured

The POL 8046 results of B & H Menthol containing 3% DAC at the expense of C57 burley showed only one qualitative difference - the experimental was harsher.

Previously, POL 8042, where 3% DAC replaced C34 burley, positive tendencies toward the experimental were seen.

## V. Menthol Blends

From studies and efforts of Project MOOG, we have recommended a blend for the Hercules (Fresh) project and a blend for the PM Blues project.

/qmm

  
C. M. Moogalian

200116445

**CHARGE NUMBER: 2100**

**PROJECT TITLE: NEW PRODUCT INNOVATIONS**

**PROJECT LEADER: H. V. LANZILLOTTI**

**PERIOD COVERED: OCTOBER, 1985**

**I. Project Vanguard**

**Objective:**

To develop a consumer acceptable nonburning smoking article.

**Status:**

Electric Cigarette: Blend and Flavor testing in progress.

Piezoelectrics: Initial subjective tests initiated.

Swept Device: Six variations of cylindrical tubes with absorptive surfaces for flavor evolution are being used for flavor screening.

**Plans:**

Increase effort on swept devices:

- a. patent implications
- b. support Flavor Development efforts
- c. collaborative efforts with other divisions
- d. optimize article design

**II. New Package Designs**

**Objective:**

To develop new and novel package designs.

**Status:**

Plastic Packs: Cigarettes received for extended use test by small internal panel. Additional packs ordered for more extensive testing. Small thermo forming device ordered for material evaluations including biodegradability.

Cricket Box: Samples of foil box, either poly laminated with tear string or foil overwrapped with center tear tape, have been shipped to Market Research for testing.

Pack Sealing: A test to determine the most desirable degree of pack sealing is being designed. Test includes packs of various Imps values, from production norm to hermetically sealed. Test to be conducted in conjunction with Packaging Studies, Flavor Development, and Analytical Research.

**Plans:**

Internal testing of plastic packs

December, 1985

Pack sealing test

February, 1986

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### III. Project Premium

#### Objective:

Develop a cigarette which will be perceived by the consumer as a premium product both in appearance and taste.

#### Status:

Initial cigarette parameters have been established and are being further developed in conjunction with Blend and Flavor Development groups.

#### Plans:

Develop "Premium" cigarette designs with regards to appearance of tipping and cigarette papers. These models will be utilized by P.E.D. in their concept and focus group evaluations of a premium cigarette product (January, 1986).

### IV. Dual-Rod Program

#### Objective:

To develop a cigarette product with dual-rod systems for enhanced taste/impact and unique flavor delivery profiles.

#### Status:

Cigarette models made with the dual-rod concept at the full flavor tar level are being analyzed for tar profiles. The initial subjectives of these models look promising with respect to first puff enhancement.

#### Plans:

Development of a cigarette at the 5-6 mg range with enhanced taste/impact on the first few puffs is continuing. These will be further evaluated on the internal panels.

### V. Microencapsulation:

#### Objective:

To develop the processing and product encapsulation technology for use in Philip Morris products.

#### Status:

H<sub>2</sub>O encapsulation: Evaluations continuing on stability studies of large (400-500 $\mu$ ) H<sub>2</sub>O capsules. OV's will be monitored on packs which incorporate these capsules on foil innerwrap at desert conditions.

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Microencapsulation Cont'd:

"Scratch & Sniff": Developing in house technology to produce small, < 50 $\mu$ , gelatin wall flavor capsules to be applied or coated on paper as in the "scratch & sniff" concept.

Crushable microcapsules: Southwest Research Institute will attempt to reformulate crushable capsules without carrageenan.

Plans:

In house encapsulation technology is on-going.

Develop working cigarette models utilizing the crushable capsules in filter designs for focus group evaluations.

VI. Flavor DotObjective:

To develop a filter flavor release system, with or without a unique end appearance.

Status:

Various filter configurations ordered from Filtrona incorporating an increased flavor solution loading.

Flavor solutions employing new PVA formula and increased menthol concentration are being tested.

Plans:

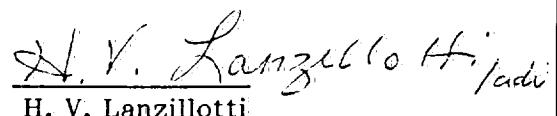
Evaluate second generation models incorporating increased flavor loading November, 1985

Accelerated aging studies December, 1985

Evaluate flavors other than menthol Continuous

Menthol consumer testing 1st Qtr., 1986

200116448

  
H. V. Lanzillotti

**CHARGE NUMBER: 2105**

**PROJECT TITLE: FILTER DEVELOPMENT**

**PROJECT LEADER: W. A. NICHOLS**

**PERIOD COVERED: OCTOBER, 1985**

**I. Filter Development - FML (W. Nichols)**

**Objective:**

To develop the technology to manufacture filters from FML polypropylene tow.

**Status:**

A production order of 168,000 filter rods was completed and shipped to India. Additional orders have been received from Brazil, Canada, and Australia.

The correlation between plugmaker efficiency and tow quality continues to be studied. Analysis of the energy required to fibrillate the film during tow production shows that minimum energy conditions cause plugmaking problems. Strip chart recordings are being used to monitor and eliminate the shipment of poor quality tow.

Installation of a newly designed crimper has produced a great change in crimp regularity. A convergent stuffer box design appears to make a more uniform crimp. Tow evaluations will begin shortly.

Installation of a new garniture and heater bar is yielding circumference control equivalent to KDF-2 standards.

Analysis of tow produced on the new extrusion line shows a trend for reduced variation in RTD. Additional sampling is being done to confirm the data.

**Plans:**

Improve plugmaker efficiency to 85% November, 1985

Prepare samples for additional market evaluation Continuous

**II. Menthol Application (G. Patron)**

**Objective:**

Assist in the introduction of menthol on foil into production.

**Status:**

Aging study of the Louisville factory produced Marlboro Menthol standard production and menthol on foil cigarettes are continuing. After seven weeks of lab storage, all the controls and two test models showed consistently uniform menthol characteristics. Menthol delivery in smoke was lowered by 0.04 mg/cig. from the original fresh cigarette values of 0.66 to 0.70 mg. Several cartons of cigarette were stored in the desert room. After two days of desert room storage, the smoke delivery was reduced down to 0.52 to 0.54 mg per cigarette while total menthol in the cigarette has remained constant. Menthol in TPM per puff for each model was characteristically the same.

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Menthol Application Cont'd

The cigarettes were submitted to PED for in-house panel testing. Test results showed that there was no significant difference in liking and on good menthol taste between the conventional production menthol and the menthol on foil cigarettes.

The second machine being built by Engineering and undergoing electrical and mechanical evaluation is complete. It is scheduled to be transferred to R&D in mid-November for our use in product testing.

Plans:

Assist Engineering and LMCP during machine start up November, 1985

Train Louisville personnel December, 1985

III. Tobacco Extrusion (R. Thesing)Objective:

To develop an extruded foam tobacco product meeting Focus Goal One requirements.

Status:

New extruded models are being fabricated for internal panel testing. An unflavored rod will be machine tipped to a series of three (3) different filter designs:

- 1) a dual filter of CA (15 mm) & Dunhill Lts. (12 mm)
- 2) a dual filter of CA (15 mm) & charcoal on tow (12 mm)
- 3) a 27 mm whistle through filter

Samples will be available the week of November 11th.

Samples subjectively evaluated with Avicel (microcrystalline cellulose), replacing a portion of the wheat starch were found to be subjectively better than the control and also improved hot collapse.

Plans:

Phase II Production Line 4th Qtr., 1985

Subjective Evaluation Continuous

2001116450

  
W. A. Nichols, a.d.v.  
W. A. Nichols

**CHARGE NUMBER: 4009**

**PROJECT TITLE: SMOKE STUDIES**

**PROJECT LEADER: B. L. GOODMAN**

**PERIOD COVERED: OCTOBER, 1985**

**I. Project Slow (J. Hearn)**

**Objective:**

Develop the technology to reduce or mask objectionable odor of sidestream smoke.

Develop a subjectively acceptable cigarette with reduced sidestream visibility.

**Status:**

The full flavor model with drastically reduced sidestream has been evaluated on the MC panel. Results showed the experimental model to be less acceptable than a commercial full flavored cigarette, even though no significant differences were seen in strength or tobacco taste. Modifications of the full flavor model have been initiated using a blend change or changes in filtration efficiencies.

The low delivery model has been made with three different after cut flavors. The cigarettes will be evaluated against the previous model that was tested on the O/C panel. Liking scores in that test were not significantly different for the low sidestream prototype and the control Marlboro Lights.

The low delivery blend has been used to test the subjective effects of Graham's salt coated on MgO paper evaluations done to date are encouraging.

Ultra Light models with 60% reduction in sidestream visibility were made with different blends. Subjective evaluations are in progress.

Several models with smaller reductions in sidestream visibility have been made with varying blends, filtration, dilution, and wrappers. They will be evaluated subjectively when the analytical data is known.

**II. Wrapper Modifications (R. Greene)**

**Objective:**

To evaluate modified cigarettes for slow burning characteristics.

**Results:**

Cigarettes have been made and smoked to examine the effects of rod density on burn rate and heat output of the cigarette. Rods were made with three percentages of expanded material at three tobacco weights each. Smoke results show the expected increases in puff count as the tobacco weight increases. Cigarettes will be smoked for burning rate and submitted to the tobacco physics group for measurements of heat flux.

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### III. Tipping Papers (R. Arthur)

#### Objective:

To investigate lip release performance from different converters.

#### Results:

Sample bobbins of EC-30436 and GSR-236M2 white tippings were received after conversion by Hermetite with .4 and .6 g/m<sup>2</sup> nitrocellulose coatings. Half of the .4 g/m<sup>2</sup> bobbins were coated on the wire side and the other half were coated on the felt side. The .6 g/m<sup>2</sup> bobbins were also coated on the wire side and felt side respectively.

The bobbins were perforated and samples taken off each bobbin for nitrocellulose extraction. The extraction results showed the samples that were supposed to be at .4 grams to be closer to the .6 gram level, and the .6 gram level samples were .6, .7, .8, and 1.0 g/m<sup>2</sup>. Strips of each were sent back to Hermetite for further examination.

Cigarettes have been made from all eight bobbins using Marlboro Lights specifications. Preliminary subjective testing will be done on both the EC-30436 and the GSR-236M2 at the .6 and 1.0 g/m<sup>2</sup> level. Testing at the .4 level will be done when the new samples are received from Hermetite.

Previous panel testing of Ecusta's 701 cork tipping on Marlboro cigarettes gave a noticeably better rating on lip release when converted by Ecusta. The EC-701 converted by Hermetite rating was not significantly different from other converters.

These two cigarette models were panel tested for lip release against the following competitive brands:

Winston	(Cork)
Kent	(Cork)
Tareyton	(Cork)
Viceroy	(Cork)
Kent	(White)
Marlboro Lts.	(White)
Winston Lts.	(Cork on White)

The results of the panel test showed that the Winston Lights was significantly better than the others with a lip sticking rating of 1.6 on a seven point scale. All the cork tipped cigarettes including the two experimental tippings were seen as equal with ratings grouped from 2.08 to 2.37. The Marlboro Lights was rated worse than the cork at 4.33, but the Kent had the worst lip sticking rating at 5.09.

B. L. Goodman  
B. L. Goodman

2001116452

CHARGE NUMBER: 4015  
PROJECT TITLE: NEW PRODUCT DEVELOPMENT  
PROJECT LEADER: W. G. HOUCK, JR.  
PERIOD COVERED: OCTOBER, 1985

I. PROJECT DATA

Objective:

To develop an adjustable tar cigarette product.

Status:

DATA test market of Concord Regular and Menthol 85's is in progress.  
Completed screening of initial Merit Select prototypes.  
DATA Menthol 85 POL extended use test completed.  
In-house, one-month extended use test (60 panelists/regular and menthol 85's) completed.

Plans:

Product Optimization	On-going
Process Optimization	On-going
Develop Merit Select Prototypes	4th Qtr., 1985

II. VIRGINIA SLIMS LIGHTS 120'S

Objective:

To develop a subjectively acceptable 120 mm version of Virginia Slims Lights Regular and Menthol.

Status:

National introduction began October 7, 1985.

III. B&H DELUXE LIGHTS

Objective:

To develop a B&H Lights Regular and Menthol with a 24.0 mm circumference for packaging in a deluxe flip top box.

Status:

Test Market began October 7, 1985 in Sacramento and Tulsa/Oklahoma City.

2001116453

IV. PLAYERS LIGHTS 25'SObjective:

To develop a 10 mg tar 85 mm and a 12 mg tar 100 mm cigarette in both Regular and Menthol to be sold as a value entry product with 25 cigarettes per pack.

Status:

Players Lights Regular 85 and 100 mm is currently in Test Market. This Test Market is scheduled to be expanded on October 7, 1985.

National introduction of Players Lights 25's in both Regular and Menthol is scheduled for December/January. Salesmen's samples were shipped October 21, 1985 and production start-up for the building of inventory for the national introduction began on October 25, 1985. It is necessary that 1.5 billion cigarettes be produced by the end of 1985.

Plans:

National Introduction	December/January
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V. CAMBRIDGE LIGHTS 20'SObjective:

To develop a 12 mg tar 85 and 100 mm value entry product in both Regular and Menthol to compete against Doral.

Status:

Cigarette specifications are being finalized for this product. The Test Market is scheduled for January 6, 1986 in Denver and Des Moines.

Plans:

Ship Completed Salesmen's Samples	November 21, 1985
Production Start-Up	December 2, 1985
Test Market	January 6, 1986

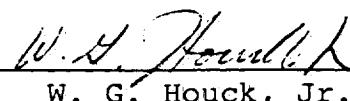
VI. PROJECT BRISTOLObjective:

To develop a 85 and 100 mm generic cigarette in both Regular and Menthol.

Status:

Several models were produced at the request of N.Y Marketing for field sampling. Nonmenthol cigarettes were produced at two tar levels, 12 and 16 mg, and in both 85 and 100 mm configurations.

Low cost blends and the foamed binder technology are being investigated for this program.

  
W. G. Houck, Jr.

2001116454

WGH:gmm

**PROJECT NUMBER: 5001**

**PROJECT TITLE: PACKAGING STUDIES**

**PROJECT LEADER: H. R. DUNAWAY** *hwd*

**PERIOD COVERED: OCTOBER, 1985**

**Objective:**

Assist New Products Directorate in evaluating new packaging concepts and products, and provide technical packaging support to Manufacturing, Manufacturing Services, Engineering, Purchasing, and Quality Assurance.

**Status/Plans:**

**A. Packaging Studies**

- Project Fresh: Completed an evaluation of nine variations of FTB blank substrate combinations to determine if any provide additional moisture loss protection. Report is in progress.
- Shelf Life Study: A shelf life study is in progress in conjunction with the evaluation of Allied Corp.'s Lifelines Inventory Management System and 3M's Monitor Mark time/temperature indicators. Use of the Lifelines System as a tool for predicting menthol migration is also being investigated.
- Project LAB: The most recent LAB samples were processed at higher temperatures in an attempt to drive off any volatiles which may have contributed to the metallic off-taste associated with previous samples. Cigarettes were packed in these boxes and stored under Desert conditions along with a soft pack control for one week. Although no metallic off-taste was detected by the Flavor Development Smoking Panel, the test cigarettes were judged to be significantly different than the controls and characterized as harsher and peppery with increased impact. Testing is currently being planned to determine if a chemical process such as fermentation is taking place.

**B. Storage Studies in Progress**

- Concord 85 Regular. Complete 11/5/85.
- B&H Deluxe Lts. Regular. Complete 11/1/85.
- Marlboro Standardization Run 2. Complete 11/7/85.
- B&H Deluxe Lts. Menthol. Complete 11/1/85.
- Players Lts. Menthol 85 & 100. Complete December 1986.
- Concord 85 Menthol. Complete 12/19/85.
- Mentholated Foam Binder. Complete April 1986.

**C. Storage Studies Completed**

- Lark Super Lights - report in progress
- Byzantium - report in progress
- Extruded Tobacco Rods - report in progress

2001116455

**2001116456**

CHARGE NUMBER: 0307  
PROJECT TITLE: Measurements Development  
PROJECT LEADER: D. R. Fox  
PERIOD COVERED: October, 1985  
DATE OF REPORT: November 6, 1985

## I. Mathematical Modelling

### Objective:

To maintain the tobacco models for filling power and cigarette firmness by incorporating new materials and measurement methods, conducting related studies of physical properties, and providing predictions based on the models.

### Status:

A simpler model expression for the firmness-density index model is currently being evaluated. The current model, a third order polynomial of hardness (a transform of firmness) and density index, is very unwieldy in practice. By restricting our data set to values in the normal operating range of the firmness instrument (excluding very soft cigarettes), a model of log firmness vs. density index fits the data quite well.

Some user interface macros were added to the spreadsheet version of the modeling system. However, since Lotus has announced new versions of Symphony and 1-2-3 that will be compatible with each other, further work is being delayed until the new versions arrive.

### Plans:

Upon review of the results with the new firmness model, a decision will be made whether to convert to the new form. Additional user interface routines will be written for the spreadsheet program upon receipt of the new software from CAD.

## II. Moisture Measurement

### Objective:

To evaluate new or improved methods for the measurement of moisture in filler and cigarettes.

**2001116457**

### Status:

Reconditioning was completed on the OV oven from the BL plant. After replacing the wet insulation and the door gaskets, temperature uniformity improved considerably. However, because we could not reach the wet insulation at the front of the oven, some front-to-back temperature and OV variation remained. Tests showed, however, no significant difference between the oven and the other two ovens in the lab and no shelf-to-shelf difference.

The oven is being returned to the BL plant, and a memo summarizing the work is being written.

Adjustment of the MEF ovens to equalize the air velocities has begun. It is hoped that this will reduce the oven-to-oven variation seen on monitor samples. An earlier test had shown considerable differences in air velocities that correlated with the OV differences.

A new oven from American Scientific is being evaluated. The design is a considerable improvement over the Precision ovens in that the air path is more uniform and there is a variable speed drive on the fan to regulate air velocity. Measurements of air velocity showed extremely good uniformity across the oven. However, the first OV results were somewhat disappointing, as the middle of the 3 shelves had significantly higher OV's. Temperature and velocity measurements indicate no reason for the difference, so the test will be repeated. One problem is that the oven is very large, taking over 200 OV cans. We have ordered additional dessicators to handle the testing load.

Plans:

Testing of the American Scientific oven will resume when the dessicators are received. The MEF ovens will be evaluated with monitors upon completion of the air flow adjustments.

### III. Materials Evaluation Facility

Objective:

To provide physical testing services (CV, OV, firmness, loose ends, etc.) to groups inside and outside R&D.

Status:

A job order request was submitted for the new RH-temperature control equipment for the laboratory. A system from Honeywell has been selected that will permit averaging multiple RH sensors, calibration of sensors, and logging of room and utility conditions.

Work has started on the programming for the computer to interface with the new FTR compacimeters. Frank McKenney, a local computer consultant, will be doing the coding. This system is expected to be operational early next year.

Final details are being resolved for the construction of a new weight selector to be designed and constructed by Development Engineering. The new instrument is badly needed to relieve the increasing demand on weight selection.

Plans:

Work will begin on the Honeywell RH system and the weight selector upon approval of funding.

200116458

CHARGE NUMBER: 0400  
PROJECT TITLE: Tobacco Properties Applications  
PROJECT LEADER: R. S. Mullins  
PERIOD COVERED: October 1985  
DATE OF REPORT: November 8, 1985

## I. Maker Optimization Program

### Objective:

Optimize cigarette makers to reduce degradation of filler and improve product quality.

### Status:

In the initial factory test of the carding comb adjustment, providing a clearance between the combs and the carding drum did not produce a significant increase in cigarette filler size. Possible reasons for this discrepancy with prior R&D test results have been identified and are being investigated. An examination of the Mark 9 hopper has revealed that when the combs are installed and adjusted according to the Molins maintenance manual, the distance between the tips of the combs and the point at which the picker removes shreds from the carding drum may vary from 9 mm to 18 mm. A preliminary test has shown that this distance significantly affects filler degradation, with the amount of 6 mesh filler at the base of the chimney increasing from 5.4% to 7.1% as the distance was increased from 9 mm to 18 mm. Furthermore, when the combs were in the closest position they could not be adjusted far enough off of the surface of the drum to reduce degradation. Additional tests are planned to verify this finding and to determine if the position of the combs significantly affects cigarette weight variation.

### Plans:

Continue investigating the effects of comb to picker and comb to carding drum spacing on filler degradation. Continue development and evaluation of the pneumatic picker.

## II. Bonded Cigarette Ends

### Objective:

Determine the effects on cigarette quality of applying binder to the ends of cigarettes. Develop a process for applying binders to the ends of cigarettes at production rates.

### Status:

A shipping test was conducted to determine if the application of binder to the ends of cigarettes reduces the amount of loose tobacco in cigarette packs or the number of shorts under the film of the packs. Cigarettes which had approximately 0.07% degraded pectin binder applied to their ends were tested on a QA shipping simulator along with untreated control cigarettes. The cigarettes to which binder had been applied averaged 0.38 shorts under the film per pack while the control cigarettes averaged 0.92. The test cigarettes also had less loose tobacco in the packs than the control cigarettes, 2.32 mg vs 3.34 mg.

### Plans:

Obtain and test an ink jet system for use in this application.

200116459

CHARGE NUMBER: 1005  
PROJECT TITLE: Primary Process Development  
PROJECT LEADER: E. G. Craze  
DATE OF REPORT: November 8, 1985

TITLE: HAMBRO DRYER (T. Skidmore)

OBJECTIVE: Evaluate the Hambro vibrating fluidized bed dryer for use in drying tobacco materials during primary processing.

STATUS: The first phase of testing to determine the operating conditions for optimum drying performance has been completed. The results confirmed that a steaming process ahead of the dryer was necessary to properly separate the cut filler and produce the desired filling power. The optimum operating conditions were used in a test to provide a head-to-head comparison between the Hambro and the Semiworks Adt dryer. The results of this test are being analyzed and will be reported next month.

PLANS: Complete the comparison of the Hambro and Adt dryer and begin testing to evaluate the Hambro for use in drying strip and stem material.

TITLE: SMALL SCALE PROCESSING (T. Skidmore)

OBJECTIVE: Develop a small scale process that will provide cigarette characteristics that are fully comparable to those of the full scale process.

STATUS: Testing of the three most promising process variations for the small scale operation have been conducted. The evaluation of the processes will be completed when all of the requested analytical and subjective test results have been received. These results are estimated to be completed by November 15.

PLANS: Complete the evaluation of the different processes and define the small scale primary processing operation.

2001116460

TITLE: MAKE/PACK OPERATIONS (D. Albertson)

OBJECTIVE: Improve the performance and efficiency of the Semiworks Make/Pack operation.

STATUS: A study was conducted to define the quality assurance requirements for the Semiworks Make/Pack operation. Based on a review of factory QA procedures, it was concluded that quality assurance could be enhanced by: 1) the addition of a QA attendant to audit the quality of the cigarettes as they are produced, and 2) minor retraining of the operators on proper QA procedures. New procedures have already been instituted to verify target weights and to inspect trays and boxes of cigarettes for quality.

PLANS: Implement the auditing procedures recommended to assure the quality of the Make/Pack operation. Establish and schedule an operator training program.



E. G. Craze

200116461

PROJECT NUMBER: 1307

PROJECT TITLE : Reconstituted Tobacco Development

PERIOD COVERED: October 1-31, 1985

PROJECT LEADER: R. G. Uhl

## I. IMPROVED SHEET PROPERTIES

### A. Objective

Improve the physical characteristics and blend performance of reconstituted sheet.

### B. Status

1. Assistance was provided to Park 500 for the production of RL test sheets containing alternate humectant systems. Products containing 4% PG and 3% PG/2.5% glycerine were made with both the TC and 150B flavor systems. Subjective screening paralleled pilot results, indicating a slight preference for 4% PG in RLTC and a stronger preference for the combination humectant in RL-150B. Packout OV specifications were adjusted to compensate for PG loss during the OV test and there were no evident sheet attrition problems at Park 500. PG loss in the tunnel dryer was 10-15%.
2. Test RCB sheets containing the same alternate humectant systems, plus a reduced level of the combined humectant (2.5% PG/2% glycerin), were made at the BL Plant. Subjective screening indicated preference for the low addition level of combined humectant. The realized PG loss through the dryer of 50% had been anticipated and taken into account. Overall humectant levels (particularly glycerin) in the test sheets were high due to the large percentage of class tobaccos in RCB feedstock and the considerable residual humectant content in final sheet (0.4% PG, 1.7% glycerin, 0.6% TEG). Both combined humectant sheets are being remade to a finished sheet specification (correcting for residual PG/glycerin).
3. Two test blends using these sheet products are being made into cigarettes at Stockton Street. Both RLTC variants will be evaluated in combination with the preferred 150B and RCB sheets. Semiworks evaluation to determine baseline data on test sheet survivability and stability was initiated.
4. Four dryer release agents were found to be subjectively unacceptable when added at recommended application levels. CEL evaporated at several temperatures from 115°F to 170°F was made into sheet and submitted for cigarette making. Test sheets were also made with zero, normal and treble levels of SEL centrifuge sludge addback.

2001116462

C. Plans

1. Provide support for additional humectant trials.
2. Complete baseline survivability/stability study on alternate humectant sheets.

II. SUBJECTIVE MODIFICATION OF RL

A. Objective

Improve or modify the subjective character of RL.

B. Status

1. Pilot production of test sheets made from pilot and Park 500 baseweb/CEL, as well as the cross-products, was completed. Cigarettes are being made to evaluate subjective differences between the tobacco substrates.
2. The second Semiworks evaluation of the RL/RCB sheet produced at Park 500 (product age = 7 months) was completed. Sieve testing, etc., is in progress.
3. A bench scale system for the continuous production of RL/RCB precooked flavor is under construction. This facility will evaluate a continuous stirred tank reactor (CSTR). Four CSTR units in series will be utilized to approximate a plug flow reactor system. Individual vessel volumes are up to 2 liters with an initial maximum throughput of 100 ml/min. Startup for system debugging will be in November.
4. Means to chemically remove scale from the Votator batch cooking system were evaluated on the bench. Best results were obtained with 10% boiling sulfamic acid. Two of the commercial cleaning agents (bases) recommended by our metallurgical consultant were moderately successful when followed by an acid wash at a less stringent temperature. In-place cleaning and retesting of the Votator system has been put on hold as the control module has been removed and reinstalled in the new CSTR facility.

C. Plans

1. Expedite evaluation of pilot/Park 500 cross-product sheets.
2. Complete data on second RL/RCB survivability test.
3. Startup and debug CSTR system.

/dbb

P. J. H.

2001116463

CHARGE NUMBER: 1503  
PROJECT TITLE: Modified Smoking Materials  
PROJECT LEADER: J. G. Nepomuceno  
PERIOD COVERED: October, 1985  
DATE OF REPORT: November 7, 1985

## I. Tobacco Extrusion

### Objective:

To develop a process for extrusion of foamed tobacco articles.

### Status:

Modifications to the filter design resulted in further taste improvements for the extruded rod prototypes. These handmade models were made with a tobacco filler section incorporated within a dual filter plug configuration. Machine-made samples are being prepared for testing with a larger panel.

### Plans:

Complete evaluation of machine-made prototypes using the modified filters with a larger in-house panel by December, 1985.

## II. Foamed Filler Binder

### Objective:

To develop a process for applying a subjectively acceptable foamed binder to the tobacco filler in order to improve coal strength, reduce loose ends and allow for weight reduction.

### Status:

Results of an internal panel test of Marlboro cigarettes with and without binders (30% licorice/1% pectin) showed no significant differences between the two models.

Because bigger improvements in cigarette firmness were shown using a more recent binder formulation (12.5% licorice/12.5% degraded pectin), Marlboro-type models with 50 milligram reductions were made for further panel testing. These cigarettes are currently being analyzed for smoke deliveries prior to panel testing.

### Plans:

Initiate panel testing of Marlboro-type models with 50 milligram weight reduction.

Produce prototypes using modified Players Lights blend for internal panel testing.

2001116464

### III. Dry Formed Rods

#### Objective:

To develop a process for producing low density cigarette rods.

#### Status:

It was shown that tobacco coated with pectin binders and then dried could be reactivated with moisture to form a cohesively bound rod. Various methods for implementing this concept on a continuous process are being investigated.

Cigarettes with binders applied at the chimney were made on the Tandem maker using forced hot air to dry the tobacco/binder system prior to the bed entering the garniture. Cigarettes are currently being analyzed for firmness.

#### Plans:

Continue evaluation of using tobacco precoated with binders for low density rod formation.

JGN/lad

2001116465

CHARGE NUMBER: 1704  
PROJECT TITLE: Supercritical Fluid Processes  
WRITTEN BY: T. M. Howell  
PERIOD COVERED: October, 1985  
DATE OF REPORT: November 7, 1985

## SUPERCritical FLUID SAMPLING

### Objective:

Develop sampling hardware and techniques that will generate accurate and reproducible results from the supercritical fluids laboratory apparatus.

### Status:

Modification design for converting the magnetically-driven mixer to a recirculation pump is complete. Preparations were made to have the assembly recertified at the original pressure rating when the modification is incorporated. The lab unit was oriented to accept the pump and sample loop.

### Plans:

Test the recirculation pump and determine optimum speed for sample flow at minimum pressure drop. Continue solubility studies for calibrating system.

## SCE FACILITY:

Operating scenarios for the supercritical fluids facility were received from UDHE and reviewed by the facility project team. Members of the team met with UDHE in Germany to negotiate the final contract terms and to discuss the technical specifications contained in the scenarios. It was decided to have UDHE issue critical breakpoints on design cost and operational aspects prior to making a decision on relaxing the system temperature limits.

2001116466

CHARGE NUMBER : 1801

PROJECT TITLE : Expanded Tobacco Development

PERIOD COVERED: September 1-30, 1985

PROJECT LEADER: G. Gellatly

I. CONVEYOR REORDERING

A. Objective

To minimize DIET filler breakage and filling power loss during reordering.

B. Status

It has been established that conveyor reordering of DIET can be achieved in about one minute without longs loss and no additional filling power loss than present cylinder reordering. This concept greatly reduces the number of conveyors required for reordering for a production facility.

Two stage reordered product was evaluated in 100% cigarettes and 30% in DBC bright cigarettes. The longer test product was shown to survive cigarette making and improve cigarette quality. Loose ends and coal drop-off of the 100% product cigarettes showed significant improvement but these parameters were not discernible in 30% cigarettes. There was no change in cigarette weight at the same firmness.

C. Plans

1. Compare the longer product of single stage conveyor reordering product with cylinder reordered product of the same filling power to confirm the results of the two stage conveyor reordered product.
2. Develop design data to support the installation of a production test facility at Cabarrus of single stage conveyor reordering.

200116467

II. UNFOLDED STRIP

A. Objective

To evaluate processing and structural characteristics of unfolded strip for improved filler length and cigarette quality.

B. Status

Two bright strip grades (G-55 & 0-9) were followed through conventional stemmery and primary processing. Decrease in strip size and the location where that decrease takes place was seen to be grade related. G-55 suffered no decrease of 1" sieve fraction during stemmery drying but lost nearly 10% during casing in the primary. Grade 0-9 lost 11% of 1" sieve fraction during conventional stemmery drying but suffered no loss during primary casing. The same degree of unfolding of 0-9 strip occurred in product samples taken before drying, after drying, and after stemmery prizeing. Unfolding of cased strip prior to cutting appears to be the most promising application point of this as there is much less risk of recurling or refolding.

Trials to unfold strip were made on static screen attached to the 3" tower steam supply to determine if the non-tower strip unfolding is feasible (250-500°F). DBC bright strip at 18-25% UV unfolded easily at temperatures between 400-500°F providing that the superheated steam flow was sufficient to fluidize the strip. Multilayer processing of strip without fluidization severely impeded strip unfolding.

C. Plans

1. Modify the static screen to allow for more precise study of conditions required for optimum strip unfolding.
2. Determine if the Hauni tunnel, Hambro and ADT dryer and fluidized bed dryers can be used for strip unfolding and if they can be modified to use superheated steam at 400°F.
3. Determine if filler from unfolded strip can improve the quality of DIET product.

  
/dbb

2001116468

CHARGE NUMBER: 1806

PROJECT TITLE: New Tobacco Processes

PROJECT LEADER: S. R. Wagoner

PERIOD COVERED: October, 1985

I. WHOLE LEAF CUTTING

A. Objective

Develop a process for cutting and separating whole leaf into cigarette filler.

B. Status

The cigarette comparison evaluating whole leaf filler produced by the D&F feeder with the pneumatic stripper was completed. In this study, cigarettes from five blends were made: 1) 100% bright strip control, 2) 100% bright leaf test, 3) Marlboro control, 4) Marlboro containing whole cut bright leaf, and 5) Marlboro containing whole cut bright leaf that was superheated in the Semiworks Hauni tunnel prior to drying. Exit the garniture, the blends made from the test filler had larger >6 mesh values than the corresponding controls: 18.4 vs 14.5% in 100% blends, and 6.7 (superheated) and 6.4 vs 5.4% in a Marlboro. However, at a constant density, the 100% bright test blend was 4.6 mm\*10 less firm than the control, while there was no difference between the Marlboro control and the Marlboro containing normally processed whole cut leaf. Marlboro cigarettes produced with the superheated whole cut leaf were slightly more firm (1.5 mm\*10) than the other Marlboro blends. At a constant firmness, there were no significant differences in loose ends between the test and control cigarettes.

C. Plans

Investigate superheating as a treatment prior to drying for whole cut leaf filler.

II. STEM TREATMENT

A. Objective

To develop methods of improving the fibrillation and subjective character of bright and burley stems.

200116469

B. Status

With consultation from Biochemical Research, a test program was designed to identify the mechanism causing the positive subjective modification produced from treating stems with cellulase. This program involves evaluating variations of the normal (50% OV, 50°C, 8% enzyme, 48 hr incubation) treatment in a stepwise manner. Thus far, shredded stems have been treated at the above moisture and temperature without the addition of enzyme for 24, 48, and 72 hr. Other variations that have been conducted are treatment at 4°C for 48 hr, and otherwise normal treatment at 24 hr. All treatments are being done with 10 lb batches, so that machine-made cigarettes can be evaluated.

C. Plans

Complete the test program described above.

III. MECHANISM STUDIES

A. Objective

To determine the mechanisms that are important to the processing of tobacco, such as attrition, drying, fluidization, etc.

B. Status

In the burley spray preparation study, blends were produced in which a common batch of burley was flavored with burley spray prepared at Stockton Street, the MC, or at R&D. Subjective and analytical investigation will determine whether the differing methods of preparation at these locations have any effect on the product.

C. Plans

Complete the data analysis from the above test.

IV. FOAMED BINDER

A. Objective

Develop a method to produce a binder for a bonded cigarette rod.

B. Status

The pectin degradation vessel, obtained from Lee Industries, was delivered and installed. Start-up and checkout of the vessel is in progress.

C. Plans

Using the vessel, assist Project 1503 in the development of a binder.

200116470

*J. R. Wagoner*

**2001116471**

PROJECT CHARGE: 1702

PROJECT TITLE: FILTRATION PHYSICS

PROJECT LEADER: R. W. Dwyer

PERIOD COVERED: October 1 - 31, 1985

DATE OF REPORT: October 28, 1985

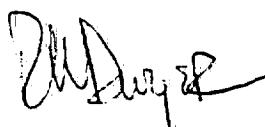
AMMONIA-SUGAR REACTION (Dwyer)

The preliminary phase of a program to determine the mechanisms of ammonia-carbonyl reactions was completed. This phase was concerned with a theoretical investigation of pyrazine formation from ammonia and  $\alpha$ -hydroxyethanal reactants. MNDO molecular-orbital calculations were performed and the lowest energy pathway for the production of  $\alpha$ -aminoethanal was determined.

SMOKE STUDIES (Cox and Morgan)

The light-extinction apparatus has been used to determine the contributions of true particulate matter and of condensed vapors to "TPM" on a puff-by-puff basis. Additionally, the average particle size of the smoke aerosol has been determined at 35 millisecond intervals during a single puff. This measurement presents us with a size profile during a puff. These techniques have been applied to low density rod samples.

/ev



2001116472

PROJECT CHARGE: 1703

PROJECT TITLE: CIGARETTE MAKING TECHNOLOGY

PROJECT LEADER: A. Robinson

PERIOD COVERED: October 1 - 31, 1985

DATE OF REPORT: October 25, 1985

TOBACCO ADHESIVE STUDIES (T. E. Majewski)

Several batch samples of sucrose solutions were made, in cooperation with Project 1806 personnel, and evaluated in the foamed injection process on the cigarette maker. The first batch of adhesive material was prepared by the reaction of calcium acetate with sucrose at ~ 95°C. The second batch by heating the sucrose solution at ~ 95°C after being made basic (pH ~ 10) with sodium hydroxide. The cigarettes are in the process of being analyzed for firmness and loose ends. Visual observation of the two cigarettes shows the one made using the sucrose/calcium acetate to hold the tobacco shreds together whereas the one made with sucrose/sodium hydroxide does not.

The minimum amount of calcium acetate needed to react/complex with sucrose to produce a coherent film on drying was found to be 6% by weight based on the amount of sucrose present. The mixture forms a stable foam with licorice.

Aluminum hydroxide was found to produce an adhesive-like material with sucrose which also produced a coherent film and produced a stable foam with licorice.

*A. Robinson*

/ev

2001116473

PROJECT CHARGE: 1706

PROJECT TITLE: TOBACCO PHYSICS

PROJECT LEADER: D. B. Losee

PERIOD COVERED: October 1 - 31, 1985

DATE OF REPORT: October 28, 1985

HEAT TRANSFER STUDIES (Tiller, Losee)

Temperature/time distribution studies on all oriental cigarettes (R. Greene) with two different wrappers and two different weights have revealed different linear burn rates in contact with a substrate as well as different peak temperatures. The sample with the lowest temperature (406°C) recorded in the substrate also had the lowest contact linear burn rate (2.1 mm/min). The free smolder linear burn rate for this sample was 2.6 mm/min. Two of these cigarettes had the same citrate wrapper, but differed in weight (833 mg versus 915 mg). These versions had the same contact-linear burn rate, free smolder mass burn rate, free smolder-linear burn rate, but slightly different maximum contact temperatures (443°C for the 833 mg model versus 454°C for the 915 mg model).

Studies relating free smolder mass burn rates to heats of vaporization and void fraction are continuing. The mass burn rate - as determined gravimetrically - of a bone dry control (~ 2% moisture) is the same within experimental error as the mass burn rate of the equilibrated control (~ 12% moisture). The free smoldering heat flux for the bone dry control is significantly higher (~ 30%) than the equilibrated control.

Linear compression at constant cross sectional area of various circumference cigarettes is being used to explore the effect of void fraction on mass burn rate, heat flux and free smolder temperature. Cigarettes treated with 5, 10, and 15% glycerin have been made for a study of the effects of heat of vaporization on free smolder mass burn rate, heat flux and temperature. Cigarettes are currently being tested for OV, circumference, static burn time and glycerin levels.

NICOTINE EVOLUTION STUDIES (Kang, Waymack)

The EG/MS technique is being used to examine tobaccos with low naturally occurring alkaloid levels. These samples, provided by R. Kinser, are being compared with the same tobaccos which have had nicotine citrate added at the 2% alkaloid level. The temperature profile for mass 84, a nicotine mass fragment, is being followed in these experiments to determine any temperature evolution differences between endogenous nicotine and a nicotine salt added to a tobacco substrate.

200116474

THERMAL ANALYSIS STUDIES (Tiller, Waymack)

A residue from laser perforating Eve tipping paper is composed of ink and lip release compound. Both materials contain plasticizers and cellulose nitrate.

/ev

DB-foolef-

2001116475

CHARGE NUMBER: 1708

PROJECT TITLE: Physical and Chemical Properties of Tobacco

PERIOD COVERED: October 1-31, 1985

PROJECT LEADER: H. A. Hartung

DATE OF REPORT October 31, 1985

### I. Effects of Additives and Environment on Tobacco

Objective: To define the physical properties of tobacco beds as a function of moisture, temperature and additives.

Status: Fifteen different tobaccos and sheet materials with various additives have been put into a test program. CV/OV testing is being done directly in a chamber at extreme conditions of temperature and humidity. Tests have been done at 110°F at RH levels of 10 and 90%.

Plans: Test at 40°F at the extremes of RH possible with the PGC chamber.

### II. Mechanical Properties of Treated Papers

Objective: To characterize experimental paper treatments intended to improve the filling power of sheet materials.

Status: The new "IMass Dynastat" Testing Machine was put into service, calibrated and checked. It worked well with experimental papers submitted by Peter Martin. Sample preparation has become the most tedious aspect of this work. Creep compliance and low speed dynamic testing has proceeded very smoothly.

Plans: Review paper data with Peter Martin. Begin testing of RL and tobacco lamina.

### III. New Plasticizers and Humectants

Objective: To unearth new materials for screening as plasticizers and humectants for smoking products.

Status: A broad literature search was run and hundreds of references were reviewed. About two dozen candidates for future screening were identified.

Plans: Complete search and review steps. Begin more detailed study of literature on candidates identified in preliminary reviews.

### IV. Reports:

**2001116476**

- (1) M.E. Counts and L.M. Trentham, "RL and RCB Impact Degradation," memo to Bob Rogers and Ron Ellis, September 11, 1985.

- (2) J.C. Crump and H.A. Hartung, "Moisture Sorption Properties of Marlboro Pack Components," Special Report 85-203, September 23, 1985.
- (3) J.C. Crump and H.A. Hartung, "Application of a Theoretical Model to Moisture Sorption of Tobacco," paper given at Tobacco Chemists Research Conference, Montreal, October 4, 1985.
- (4) M.E. Counts, K.A. Cox, B.C. LaRoy and P. Martin, "Pressure vs Volume Properties of Tobacco Networks," talk at Philip Morris Science Symposium, October 18, 1985.

*H.A. Hartung*

2001116477

CHARGE NUMBER: Project 1720

PROJECT TITLE: Physiochemical Morphology

PERIOD COVERED: October 1-31, 1985

PROJECT LEADER: E. Thomas

DATE OF REPORT: October 28, 1985

Objective: To determine the biochemical and biophysical properties of chloroplast submembrane preparations with respect to oxygen evolution and elucidate the degradation pathways of chloroplast proteins as a function of senescence. (V. Baliga and H. Nakatani)

Status: A procedure was developed to decrease the amount of LHCII component in triton-derived, PSII oxygen-evolving preparations. Thirty mM octyl-glucopyranoside lowered the levels of LHCII in PSII pellets derived from ultracentrifugation. Treatment with urea was also tested, and, while it removed the 33 kDa polypeptide, there was little effect on amount of LHCII. Electron transport and chlorophyll a/b ratios were measured on immature, ripe, and senescent leaves from Coker 319 tobacco. It was determined from these tests that both PSI and PSII reaction centers of the senescent leaf were still functioning, but that their antenna systems were degraded.

Plans: Studies will continue in the investigation of the differences in photosynthetic electron transport between green and senescent tobacco leaves. In another study, whole PSII particles will be separated from thylakoid membrane extracts using detergents other than triton. The oxygen evolving activity from purified components of PSII will be compared to the activity of intact photosynthesizing particles.

Objective: Study the physical and chemical properties of green tobacco and relate them to the mechanical properties of cured leaf. (E. Taylor, E. Thomas, J. Lyle, P. Echlin)

Status: Tobacco protoplasts at different stages of cell wall regeneration were studied by LM and SEM. In the LM studies Calcofluor, a cellulose-specific fluorescence stain, was used to visualize the relative degree of cell wall regeneration for a set of tobacco protoplasts. These protoplasts had been collected at different times during cell wall regeneration and preserved in 1% glutaraldehyde. Different preparation schemes were studied for their ability to preserve the cell wall features of tobacco protoplasts. These have included critical point drying and freeze-drying. Frozen-hydrated protoplasts were also prepared and examined by SEM. Individual features of the cell wall could not be distinguished because of the presence of surface ice.

Plans: Studies will continue on the imaging of the surface morphology of tobacco protoplasts using the SEM and TEM. Methods to remove surface water before freezing will be examined. In addition, freeze-dried samples prepared from frozen-hydrated protoplasts will be used with the higher-resolution STEM techniques.

2001116478

SERVICE WORK: The particle size distribution of a sand sample was studied at the request of J. Lewis. The diameter of the sand, isolated from field tobacco, was determined using polarized light microscopy followed by video image analysis. In another request, image analysis techniques were used to determine the length histograms of tobacco shreds cut from different sizes of burley strip. This work was done in collaboration with the Engineering Department. At the request of T. Skidmore, MKS filler that was dried in either the ADT or the Hambro dryer was examined by SEM for differences in surface features. No differences were found.

- Eddie Horner

2001116479

CHARGE NUMBER: Project 1730  
PROJECT TITLE: Plant Tissue Research  
PERIOD COVERED: October 1-31, 1985  
PROJECT LEADER: L. Weissbecker  
DATE OF REPORT: October 29, 1985

I. Objective: Produce tobacco plants with significantly reduced leaf Cd levels (Shulleeta, Spriggs and Weissbecker).

Status: Plants were grown aseptically in various configurations. These were:

1 liter Erlenmeyer flasks containing liquid growth medium and glass beads for plantlet support.

Magenta GA7® containers containing liquid growth medium and pyrex glass wool for plantlet support.

Plant Cons® containing agar solidified growth medium for plantlet support.

Plant Cons® containing sulfur free agarose solidified growth medium for plantlet support.

Plant Cons® containing liquid growth medium and glass beads for plantlet support.

From limited data, it appeared that there was a tendency for larger plants (based upon leaf dry weight) to have a higher concentration of leaf Cd ( $\mu\text{g/gm}$  dry weight) for plants grown in Erlenmeyer flasks and Magenta GA7's.® The opposite tendency seemed to occur in plants grown in agar or agarose solidified media (i.e. Cd concentration appeared to decrease with increased leaf weight). No trend was apparent in plants grown in Plant Cons® in liquid medium supported with glass beads. A second experiment with Plant Cons® and glass beads showed the same lack of any trend. It is assumed that the Cd concentration should be independent of leaf dry weight but vary based upon plant uptake only. Agar samples within and outside the plantlet root mass were submitted to Ms. P. Grantham of the Analytical Division for Cd analysis. The objective was to determine if the agar was limiting the diffusion of Cd to the roots and hence producing an "apparent" decrease in the leaf Cd content. The results were inconclusive; one set showed more Cd near the roots than away from the roots while the second set indicated that there was less Cd near the roots than away from the roots.

Plans: Use Plant Cons® containing liquid growth medium with glass beads for plantlet support as the standard method of exposure. **2001116480**

II. Objective: To produce diploid plantlets from haploid Coker 319 plantlet material (Shulleeta).

Status: Plantlets have been obtained from pieces of mid-vein from haploid Coker 319 plantlets. Some shoots have been generated from haploid Coker 319 roots.

Plans: Analyze the roots of regenerated plantlets for their chromosome number.

III. Objective: Produce Coker 319 flue-cured tobacco leaves with lowered nicotine content (Sanders and Weissbecker).

Status: A small, select panel smoked cigarettes made from Coker 319 scions grafted onto roots of tomato plants. The filler nicotine concentration was below the analytical detectable level (<0.3% alkaloids). The panel felt that the smoke had a full flavor but lacked any feel sensations.

Plans: A report will be issued as soon as all the data has been received.

IV. References

K. Sanders - notebook No. 8165.  
M. Shulleeta - notebook No. 8228.

L. Weissbecker

2001116481

CHARGE NUMBER: Project 1901  
PROJECT TITLE: Biochemical Modification of Tobacco  
PERIOD COVERED: October 1-31, 1985  
PROJECT LEADER: D. M. Teng  
DATE OF REPORT: October 30, 1985

1. Direct Utilization of Tobacco

Ripe bright tobaccos of mixed stalk position (control and Ethrel-yellowed) which were dried in one of the three ways: 1) freeze-dried, 2) semi-work dried and 3) flue-cured, were processed. Some of the samples were expanded by the DIET process at the Pilot Plant. All the samples are being evaluated for chemical compositions, biochemical attributes and subjective responses.

2. Tobacco Samples From Different Growth Stages

Mid-stalk fresh tobacco leaves were secured from different stages of growth. All the samples were freeze-dried and cut. Cigarettes are being made for subjective and biochemical evaluations.

3. Burley Tobacco Curing Studies

Inhouse air-curing of burley tobacco under different moisture levels and high temperature-high humidity treatment are on-going. Curing will be terminated in another four-weeks.



2001116482

CHARGE NUMBER: Project 1902

PROJECT TITLE: Tobacco Microbiology

PERIOD COVERED: October 1-31, 1985

PROJECT LEADER: D. K. Chadick

DATE OF REPORT: October 30, 1985

1. Definition of Spoilage:

Objective: Outline the succession of events during the deterioration of SEL and RL sheet.

Status: A positive control sheet has been created using a low inoculum of our group of four known spoilage bacteria. The four cultures were grown for four hours in a suitable medium and inoculated onto sterile RL/TC sheet in a 1:1:1:1 ratio. The starting level of organisms on the sheet was  $1.9 \times 10^5$  bacteria/g of sheet. This number is considerably lower than the inoculum level ( $\sim 1.0 \times 10^8$  bacteria/g) used in other positive control experiments. By using a lower amount of organisms, we were able to observe the effect on the sheet in a more realistic manner; i.e. "bad" sheet (based on acetic acid amounts) that we have extracted for organisms and counted seldom has had more than  $1.0 \times 10^6$ - $1.0 \times 10^7$  bacteria/g of sheet.

The inoculated sheet was stored at 37.5°C and 80% RH for six days. Samples were extracted for bacterial counts and acetic acid analysis at 0 time (just prior to inoculation) and on days 3, 4, 5 and 6. The organism count ( $2.3 \times 10^7$  bacteria/g of sheet) was still increasing at day six. This was in contrast to previous experiments which employed larger amounts of inoculum. In these latter series of experiments peak organism number was achieved in 4-5 days. The acetic acid values for the current experiment are not yet available.

Plans: Repeat this type of positive control sheet experiment using a lower level of inoculum for a longer period of time. At the request of Chris Kroustalis, we also plan to inoculate sheet with the larger number of bacteria, incubating at 37.5°C and 80% RH and withdrawing daily samples for Chris Kroustalis. Chris will use these samples for a variety of analyses and plate counts will also be done.

2. TEG Solvent Replacement in SEL:

Objective: Evaluate suitable solvents to replace TEG in SEL. The selected solvent should be compatible with the propylparaben preservative and act as a humectant in the finished sheet.

Status: Two experiments have been performed using different solvents/humectants with and without paraben in SEL, made without class tobacco in the C Pilot Plant, that had been inoculated with the same group of four known spoilage bacteria (KSB) used in the positive control sheet experiments. The bacteria were used in 1:1:1:1 ratio. The first test

200116483

involved the use of propylene glycol(PG), glycerin (G), sorbitol (S), and triethylene glycol (TEG) with and without paraben. The solvent/humectant levels remained constant at 10% (v/v) and 20,50, and 100 ppm propylparaben were used where necessary. Sterile SEL was inoculated with the appropriate amounts of the solvents/humectants and paraben to yield the above mentioned amounts and 10% (v/v) bacterial mixture was also added (final concentration=9x10<sup>7</sup> bacteria/ml). Two controls were also included in this study, one flask contained only sterile SEL the other contained sterile SEL with a 10% inoculum of bacterial mixture. The flasks were incubated at 37.5°C and 150 rpm for five days. Samples were removed daily for bacterial plate counts, acetic acid analysis, and propylparaben analysis.

The following results were observed after 5 days of incubation:

1. The solvent controls alone affected the growth of the bacterial mixture compared to the control (bacterial mixture only). PG decreased the bacterial counts in 24h to 2.5x10<sup>4</sup> organisms/ml. As expected, not much acetic acid was produced in the culture containing only organisms and PG.
2. Sorbitol showed a slight decrease in the bacterial counts at the five day period (3x10<sup>6</sup> bacteria/ml) with an increase in acetic acid production (325 ppm) but not to the level as seen in the control which contained the microbes only (at time 0 acetic acid was about 15 ppm and increased to 450 ppm after five days incubation). Glycerin alone caused a decline in the bacterial counts to levels similar to that obtained with PG. However, the culture containing only glycerin required five days to effect this decrease while PG took only one day. Thus, as expected, the glycerin culture showed elevated acetic acid production (375 ppm after five days). Finally the flask with TEG and organisms actually exhibited an increase in bacterial concentration for the five day period (7x10<sup>8</sup> organisms/ml versus 1x10<sup>7</sup> bacteria/ml for the culture without any solvent at the end of five days of incubation). However, the TEG flask showed a decreased level of acetic acid (250 ppm) versus the control (450 ppm). All of the above mentioned results were probably a direct result of the high solvent concentration (10% v/v) employed in these experiments.
3. As expected, for the most part the addition of 20,50, and 100 ppm propylparaben with the solvents/humectants had little effect on the growth of the bacteria after five days. There was some initial decrease in the bacterial counts after 24 hrs with all levels of paraben in all the experiments;however, bacterial recovery occurred shortly thereafter. Also, after five days the acetic acid levels were slightly lower than the inoculated SEL control (200-350 ppm). This observation on the effect of propylparaben is not surprising since all current literature stresses that propylparaben offers a bacteriostatic and not a bactericidal effect. However, the results with the paraben could be slightly misleading since 10% levels of the solvents/humectants were used. In the next group of experiments these solvent/humectant levels were employed at more realistic amounts.

**2001116484**

A second experiment similar in nature to that previously mentioned was conducted using 1.5% TEG, 1.8% PG, and 1.8% PG + 1.5% glycerin with and without propylparaben. The levels of paraben were as follows: 100, 200, 300 and 400 ppm. All solvent/humectant percentages are on a (v/v) basis. The

same type of sterile SEL was used as above and each flask was inoculated with a 10% inoculum of the same known group of four bacteria ( $1.9 \times 10^7$  bacteria/ml) in the same ratio as previously mentioned. The flasks were incubated four days at  $37.5^{\circ}\text{C}$  at 150 rpm. The same two controls used in experiment I were also used. After four days of incubation the inoculated control contained  $8 \times 10^5$  bacteria/ml. A four day experiment was chosen because it was noticed in the first experiment, while using less paraben, that the effect, if any, was complete by day four.

Bacterial data collection for this experiment is complete and is being tabulated, samples have been submitted for fatty acid analysis, and the results will be reported next month.

The levels of solvents/humectants (with the exception of the first experiment) and propylparaben used throughout both of these groups of experiments were chosen, bearing in mind, the amounts of these compounds that can be realistically used at Park 500 production facility (1).

Plans: Repeat this study after all the current data has been analyzed and discussed with the appropriate personnel.

### 3. Humectant Replacement Trials in C Pilot Plant:

Objective: Store RL/TC and RL/150B sheet that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: Samples of the acceptable sheet are currently being stored in our environmental rooms at  $37.5^{\circ}\text{C}$  and  $25^{\circ}\text{C}$  with 80% RH maintained at both temperatures. The sheet will be analyzed for bacteria, yeasts, and mold at 1, 2 and 4 week intervals and at 2 and 3 months. Acetic acid analysis will also be done on a monthly basis.

Plans: This is an ongoing study and the data will be reported monthly, based on the storage time of the various sheets.

### 4. Humectant Replacement Trials at Park 500:

Objective: Store RL/TC and RL/150B sheet that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: The sheet collection was completed on October 24th, and the samples will be treated as mentioned in item 3. In addition to the storage study done in our environmental rooms, hogsheads of sheet will be stored in a warehouse. At appropriate times, they will be opened and samples will be removed and analyzed as stated in item 3.

Plans: As previously mentioned in item 3.

### 5. Humectant Replacement Trials at the BL Plant

Objective: Store RL/RCB that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

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Status: Sheet has been collected and storage will begin this month as previously described in item 3.

Plans: As previously mentioned in item 4.

6. Mold Analysis of a Silo in OC Containing DBC Bright Tobacco:

Objective: At the request of Ed Craze (OC), samples of DBC bright tobacco in a blend silo in the semi-works were analyzed via plate counts for mold.

Status: A memo was issued and recommendations were made to prevent a future occurrence of the molding problem (2).

7. Bacterial Analysis of an Adhesive With and Without the Addition of Licorice:

Objective: At the request of Sue Wrenn, two samples of a sucrose-Ca acetate binder with and without the addition of 0.75% licorice were analyzed for bacterial contamination. This request was made in an attempt to explain a loss in stability of the adhesive with licorice. A sample of the licorice was also analyzed for bacteria.

Status: A memo has been issued and recommendations were made to prevent future possible contamination of the adhesive by the licorice (3).

References

1. Personal communication with B. Semp.
2. Chadick, D., Mold Analysis of a Silo Containing DBC Bright Tobacco. Memo to J. Nguyen, 1985, 24, October.
3. Chadick, D., Bacterial Analysis of a Sugar Adhesive With and Without the Addition of Licorice. Memo to S. Wrenn, 1985, 14, October.



2001116486

CHARGE NUMBER: Project 1904

PROJECT TITLE: Tobacco Physiology and Biochemistry

PERIOD COVERED: October 1-31, 1985

PROJECT LEADER: I. L. Uydess

DATE OF REPORT: October 29, 1985

Objective: To establish the time course and biochemical changes characteristic of tobacco leaves at various stages during senescence.

Status:

I. Immunochemical Phytohormone Assays (In collaboration with B. Davies).

Aqueous, 80% methanol extracts of greenhouse and field-grown, green leaves, and greenhouse-grown, senescing leaves of Coker 319 tobacco have been used in the initial testing of an enzyme-linked, immuno-sorbant assay ("ELISA") kit from Idetek, Inc. for abscisic acid (ABA). Although the values obtained were unexpectedly high and far above the range of the standards employed due to the inadvertent use of overly concentrated extracts (low values were initially expected), the preliminary indications are that these kits can be used successfully in the analysis of abscisic acid in our tobacco leaf samples. Similar procedures will be employed with regard to the evaluation of indole acetic acid (IAA) in these, as well as in other tobacco leaf extracts over the next two months.

II. Enzyme Assays and Associated Methodologies.

The methodology for the analysis of carboxypeptidase B using a commercial enzyme preparation has been established, and is currently being employed to evaluate carboxypeptidase B activity in tobacco leaf extracts. However, as in the cases of endopeptidase and carboxypeptidase A, no activity has been demonstrated in either frozen greenhouse or field-grown leaves, or fresh greenhouse leaves using several different extraction procedures. The leucine aminopeptidase (LAP) assay, on the other hand, continues to demonstrate the activity of this exopeptidase in all of the tobacco extracts examined. Project 1904 (and associated personnel) are currently investigating the basis for this problem along several lines including:

1. Concentration of Extracts: Is this a concentration problem with respect to an extremely low level of some of these enzymes in the mature and/or senescing leaves of the tobacco cultivar being evaluated?

Results: Two experiments were conducted in which tobacco leaf extracts were concentrated approximately 3 and 10-fold using an Amicon membrane filtration device. However, no activity was subsequently realized in either of these concentrated extracts for endopeptidase. Additional

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experiments utilizing ammonium sulfate and membrane concentration procedures are in progress.

It is also possible that Coker 319 (whether field or greenhouse grown) has extremely low levels of these two groups of enzymes, or alternatively, that these particular tobacco enzymes have different specificities with regard to what is (or what is not) an acceptable substrate (the substrate specified for the commercial enzyme preparation is generally employed). To explore these two possibilities, a variety of tobacco cultivars will be examined, along with alternative substrates.

2. Protease Inhibition: Are some of these problems due to the inactivation of the targeted tobacco enzymes as a result of the use of protease inhibitors during our extraction procedures (inhibitors of protein degradation normally used to protect such enzymes)?

Results: Two experiments were conducted employing a commercially available, yeast endopeptidase (used normally as our control), as well as two plant-specific proteases (bromelain and papain) thought to be more homologous to the endogenous tobacco enzyme than that isolated from yeast. In each case, the protease inhibitors currently being used (leupeptin and PMSF) were added to one of two duplicate tubes containing one of each of the above-mentioned yeast or plant enzymes in order to assay for the possible inhibition of these plant specific enzyme activities. Leupeptin, but not PMSF, was found to inhibit the activity of the two plant proteases, while PMSF (but not leupeptin) was found to inhibit the yeast enzyme. Thus, while this question has not been completely resolved by these initial experiments, the results do suggest that further work in this area is required, and that caution must be exercised when planning to utilize such protease inhibitors in the evaluation of plant protease enzyme activity.

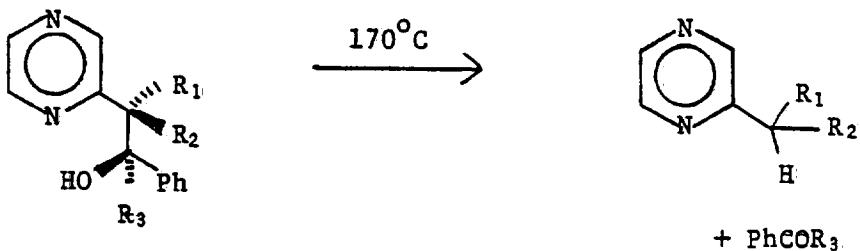
Plans: To continue testing for enzyme activities in tobacco extracts prepared from material grown in the greenhouse or in the field under various conditions.

*—Jan Hydes*

2001116488

CHARGE NUMBER: 2500  
 PROJECT TITLE: FUNDAMENTAL CHEMISTRY  
 PROJECT LEADER: Y. Houminer  
 PERIOD COVERED: September 1, 1985 - October 31, 1985  
 DATE OF REPORT: November 1, 1985

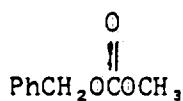
We have completed<sup>1</sup> a study on the solution thermolysis of a series of pyrazineethanols. The reactions kinetics were followed by n.m.r. and rate constants, at 170°C in diglyme-d<sub>14</sub>, were measured. The results shown in the table indicate a significant steric effect for methyl substituents at the  $\alpha$ - and  $\beta$ -positions. Preliminary MOMM calculations gave good correlation between steric strain release and rate constants<sup>2</sup>.



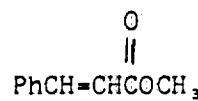
<u>R<sub>1</sub></u>	<u>R<sub>2</sub></u>	<u>R<sub>3</sub></u>	<u>k sec<sup>-1</sup> x 10<sup>6</sup></u>
H	H	H	1.81
H	CH <sub>3</sub>	H	5.44
CH <sub>3</sub>	H	H	4.61
H	H	CH <sub>3</sub>	8.91
CH <sub>3</sub>	CH <sub>3</sub>	H	53.77
H(CH <sub>3</sub> ) mixture of diastereomers	CH <sub>3</sub> (H)	CH <sub>3</sub>	18.57
CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	426.50

2001116489

We continue our work in the area of coumarin substitutes. It has been suggested that a carbonate and an  $\alpha, \beta$ -unsaturated ester may have similar odor properties. Indeed, carbonate I was synthesized and its odor properties were found to be very similar to that of methyl cinnamate (II).

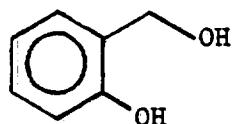


I



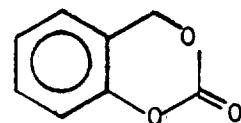
II

The above observation was extended to the area of coumarin substitutes. Treatment of the diol III with phosgene gave the desired carbonate IV. The latter has an aroma very similar to that of coumarin<sup>3</sup>.



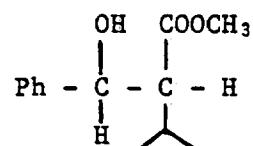
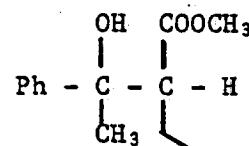
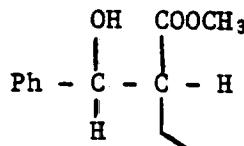
III

$\xrightarrow[\text{base}]{\text{COCl}_2}$



IV

Several new  $\beta$ -hydroxy esters were prepared by reacting the required enolate anion of an ester with either benzaldehyde or acetophenone<sup>4</sup>. The following compounds were obtained, each as a mixture of diastereomers which was separated by LC. This completes the synthesis of 18 different  $\beta$ -hydroxy esters which will be tested for steric effects in their thermolysis.



An attempt to react the enolate anion of methyl 2-methylbutyrate with isobutyrophernone gave the corresponding propiolactone instead of the expected  $\beta$ -hydroxy ester. This unusual behavior is now under investigation<sup>5</sup>.

A novel extension of a known pyrrole synthesis from the reaction of diethyl aminomalonate and 1,3-diketones to that of diethyl aminomalonate with either malondialdehydes or  $\beta$ -chlorovinylaldehydes was explored. Several pyrrole derivatives were prepared via these new routes and will be tested as potential flavorants<sup>5</sup>. Some of these pyrroles are believed to be present in smoke<sup>5</sup>.

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Using the refined M0MM parameters, we have recalculated, reanalyzed, and now finalized the steric effect parameters for congestion at the nitrogen atom of pyridines, pyrazines and other aza heterocycles. An excellent correlation was obtained between these steric parameters and experimental kinetics data. The musk-like character of several odorants (ArCOR, where R = H, Me, Et, i-Pr) correlates with the steric energy model derived from the above methodology<sup>2</sup>.

Together with Dr. A. Kassman, we have developed a mathematical relationship which describes the kinetics of the process of warehouse filler treatment with phosphine. Comparison of this mathematical expression with experimental data indicates that phosphine does diffuse out of the polyethylene "containers" and the time it takes for this diffusion to occur can now be predicted<sup>2</sup>.

Odor profiling for a series of coumarin analogues, including the two thio-coumarins recently prepared, has been completed. Analysis of the data enables us to determine which compound may be the best substitute for coumarin<sup>6</sup>.

Analysis<sup>7</sup> of fatty acids in different types of Oriental tobaccos has been carried out in order to determine the quality of the Oriental tobacco. The results suggest that the ratio of 2-methylbutyric acid to 3-methylvaleric acid may be one parameter that can be used to determine Oriental tobacco quality<sup>6</sup>. Other parameters could be relative concentrations of scleral and abienol.

Preliminary odor evaluation of precook samples suggests that it may be possible to screen for acceptability by using this method<sup>6</sup>.

References:

1. Y. Houminer
2. J. Seeman
3. K. Podraza
4. H. Secor
5. J. Paine
6. E. Southwick
7. F. Hsu

*X. van Houmer*

2001116491

CHARGE NUMBER: 2501  
PROJECT TITLE: SMOKE CHEMISTRY  
PROJECT LEADER: R. H. Newman  
PERIOD COVERED: October 1 - 31, 1985  
DATE OF REPORT: November 5, 1985

#### IR CAMERA

The IR camera has been serviced as part of the maintenance and to correct a drifting problem in the electronics. The camera has been remounted on its translational stage and set to view the two new calibrated temperature sources. Calibration of the camera video recorder system is in progress. The results will be analyzed with the image processor and included in the program for measuring the temperature profile of cigarette coals.

#### NEUTRON RADIOGRAPHY

Line density measurements were repeated on two Winston Box and two Winston 85mm cigarette samples. The data has been reduced to graphic form and is being analyzed. A memo will be issued shortly.

#### LIMS

The Perkin-Elmer chromatography data system is essentially ready. The last few instruments are currently being interfaced and a short training course is planned for the near future. A fairly extensive on-line help facility has been developed and a new terminal driver installed which automatically disconnects the network session at logout. Three new printer/ plotters were installed and their functionality confirmed on the system. These will give users local hardcopy support for plots and reports.

#### SMOKE ANALYSIS

A sample of menthol purified by R. Izac to be supplied to C. Lilly was analyzed by capillary gc using simultaneous detection by FID, NPD, and FPD. The menthol was determined to be 99.4% pure. No peaks were found using the NPD or FPD, indicating the absence of compounds containing nitrogen or sulfur. The FID chromatogram showed the presence of 0.3% of an early peak presumed to be hexane (used in the purification) and two other small impurity peaks.

**2001116492**

The total smoke distribution of <sup>14</sup>C-nicotine labelled cigarettes was completed. The data is being analyzed. The mainstream pad, sidestream pad and butt extracts were given to R. Izac for analysis for nicotine.

## RADIATION SAFETY

Radiation safety procedures were completed this month including all required wipe tests and were found to be within tolerances.

## COUNTING EQUIPMENT

The Tennelec ratemeter, used in conjunction with a Spectrum Filter and Amplifier does an adequate job on small to medium size peaks. This system, however, does not appear to be a totally acceptable system to provide an analog output to the LIMS. It can perhaps be employed as an auxillary visual aid in comparing mass to activity peaks. Plans are to set up an Ortec counting system based on their new computer controlled nim-electronics (CCNIM) with a Model 974 Quad counter/timer which can be computer controlled (cc) or manually operated. This equipment will provide a dual counting system for the Siemans. Since the Berthold counter tubes and pre-amp/amp must be used in the flow through systems, adaptations must be made on power supplies and interfaces. This work is proceeding.



2001116493

2001116494

CHARGE NUMBER: 1101  
PROGRAM TITLE: ENTOMOLOGICAL RESEARCH  
PERIOD COVERED: OCTOBER 1 - 31, 1985  
PROJECT LEADER: D. L. FAUSTINI  
DATE OF REPORT: NOVEMBER 6, 1985

## I. CIGARETTE BEETLE PHYSIOLOGICAL STUDIES

### A. Field

Testing has continued in the warehouse evaluating several candidate pyrethroid space sprays. One particular spray is currently being evaluated at various concentrations to obtain dose-response data. The supplier of the insecticide has been contacted and requested to submit analytical data regarding breakdown products and/or residual levels. R&D anticipates making a recommendation for its use by December, 1985.<sup>1</sup>

It has been shown when export cut-filler which is packaged in polyethylene bags and fumigated with PH<sub>3</sub> for the required 96 hour period, the PH<sub>3</sub> becomes "trapped" within the bag long after the source of the gas has been exhausted and removed. An additional 30 days are added to the fumigation to allow the PH<sub>3</sub> to evolve from the exposed bags. The additional time creates problems in shipping/handling since APHIS only allows a 30 day grace period for the issuance of a Phytosanitary Certificate at the conclusion of the 96 hour fumigation. Therefore, a study was initiated to determine whether the phosphine that remains within the polyethylene bags represent an individual fumatorium for the control of the CB and to establish the length of time required to achieve mortality. If the study proves successful then APHIS will be approached for an extension for more time on the Phytosanitary Certificate.<sup>2</sup>

### B. Laboratory

Studies have continued on the attractive properties of tobacco molds by the CB. Replication data are being gathered in a dual-choice pitfall bioassay chamber.<sup>3</sup>

Several double-faced adhesives were evaluated as candidates to trap CB in an R&D designed pheromone trap, none of which were found to be satisfactory. Glue products that can be directly applied to a cardboard surface are now being calculated.<sup>4</sup>

## II. ASSISTANCE TO OTHERS

One hundred percent CB mortality was achieved at the 20th St. conditioners used for phytosanitary certification of export blended tobacco.<sup>5</sup>

**2001116495**

III. REFERENCES

1. Faustini, D. L. Notebook No. 7746, pp. 122-125; 128.
2. Faustini, D. L. Notebook No. 7746, pp. 126-127.
3. Minor, M. F. Notebook No. 7197, p. 179.
4. Drew, S. Notebook No. 7850, pp. 202-203.
5. Deubler, R. C. Memo to D. T. Wagner. Documentation of Beetle Kill at 20th St. 1985 October 3.

D. L. Faustini (so)

2001116496

CHARGE NUMBER: 1620  
PROGRAM TITLE: ELECTROPHYSIOLOGICAL STUDIES  
PERIOD COVERED: OCTOBER 1 - 31, 1985  
PROJECT LEADER: F. P. GULLOTTA  
DATE OF REPORT: NOVEMBER 1, 1985  
WRITTEN BY: C. S. HAYES

## I. EVENT-RELATED POTENTIALS (ERPs)

### A. Difference Testing Employing Cognitive ERPs

Experiments utilizing the cognitive ERP paradigm were initiated employing auditory stimuli in order to provide a quick means for: (1) verifying the relationship between the cognitive component of the ERP and stimulus discriminability and (2) investigating variables (e.g., laterality effects, response localization and polarity, etc.) which may influence the recording, measurement and evaluation of the cognitive component in smoke and flavor discrimination experiments.

To date, four subjects have been presented with pairs of tones that vary in discriminability (easy vs. difficult).<sup>1</sup> Results from these experiments are in agreement with findings from previous flavor experiments. That is, the cognitive component (referred to as the P300 in auditory experiments) elicited to the easy discrimination is larger than the component elicited to the more difficult discrimination, confirming that a relationship does exist between the size of the cognitive component and discriminability. Additionally, mapping of the responses has provided insight into where in the brain maximal cognitive responses are occurring and via FFT analyses of the data, information regarding the frequency spectrum of the component has been gained. Experimentation in this direction will be continued on an "as needed" basis to further explore variables which may be pertinent to the results of flavor and smoke discrimination experiments.

### B. Nasal Event-Related Potentials (NERPs): Smoke Studies

Design modifications were made to the new olfactometer/smoke delivery system resulting in an increase in the airstream flowrate for better stimulus delivery and ERP recordings. The problem with residual odors lingering in the olfactometer airstream during inter-stimulus-intervals was, however, not corrected, and smoke stimuli as currently delivered are not of sufficient volume to elicit good ERPs. These problems should be corrected within a week, at which time smoke studies investigating differences between smoke from DBC Bright and Burley cigarettes as well as smoke from other cigarette types and blends will continue.

**2001116497**

CHARGE NUMBER: 1620

-2-

NOVEMBER 1, 1985

II. THE EFFECTS OF CO<sub>2</sub> ON CIGARETTE SMOKE FLAVOR

Work on this study continues. One additional subject has completed discrimination training and is currently being retested. Training is nearing completion in two other subjects.

III. REFERENCES

1. Wannamaker, I. Notebook No. 8249, pp. 37-193.

*C. A. Hayes*

2001116498

PROJECT NUMBER: 1740  
PROJECT TITLE: Flavor Components of Tobacco and Filler  
PROJECT LEADER: C. S. Kroustalis  
PERIOD COVERED: October, 1985

## I. FLAVORS

A. Several CSTR cooked flavors were profiled for volatile pyrazines. The profiles were similar to normal precook samples.

B. Kretek cigarettes were analyzed for eugenol and nicotine deliveries to mainstream smoke. Djarum delivered 20% of the eugenol and 14.5% of the nicotine to mainstream. Gudang Garam deliveries were 22% for eugenol and 23% for nicotine. Both cigarettes had high puff counts (18 to 22 per cigarette).

C. Flavor "cigarettes" were analyzed for flavorants in the Dacron plug. The majority of the components were nicotine alkaloids with nicotine being the highest in concentration. The menthol version contained lower concentrations of alkaloids and menthol of comparable concentration to nicotine.

D. Guatemalan menthol was compared to natural menthol for purity and isomer ratios. Both met specifications although menthone was higher in concentration in the test sample.

E. Three types of an AC component were profiled. The proposed substitute was significantly different than the control.

F. Theobromine was determined for the Marlboro standardization program and in several menthol brands. Only Salem contained theobromine indicating that the other competitive menthol brands do not contain chocolate.

## II. OTHERS

A. The screening of warehouse RL for volatile fatty acids has continued. Approximately 400 samples have been analyzed during this period. In addition, Pilot Plant and Park 500 samples with substitute humectants were screened for volatile fatty acids.

B. Pilot Plant RL of low, medium and high acetic acid levels were extracted and analyzed for indole and skatole. There were no detectable levels present of either compounds. The lower limit of detection was 0.5 ppm. However, there were quantitative differences in butanediols and several other compounds which will be identified. To determine whether some other marker for spoilage exists, D. Chadick has inoculated autoclaved RL with bacteria isolated from SEL and stored it at 80% R.H. and 37 degrees C for a week. Samples were taken daily and they are currently analyzed.

C. Three customer complaint samples were analyzed this period.

2001116499

D. Samples from outside the Allegheny warehouse fumigation chamber were analyzed for phosphine. Although the odorant with phosphine was detectable, the concentration found was 0.02 ppm.

III. Presentation and MEMOS

1. C. S. Kroustalis, "DEG in German Marlboro and Reconstituted Sheet Materials," Richmond Meeting Presentation, October 8, 1985.
2. C. S. Kroustalis to T. S. Osdene, "Kreteks," October 14, 1985.
3. C. S. Kroustalis to E. Wickham, "GC/MS Analysis of Favor," October 14, 1985.
4. C. S. Kroustalis to R. D. Carpenter, "B&H Deluxe Ultralights 100's (CC #85049)," October 14, 1985.

*C. S. Kroustalis*

2001116500

PROJECT NUMBER: 1752  
PROJECT TITLE: Optical Spectroscopy of Tobacco and Smoke  
PROJECT LEADER: R. A. Fenner  
PERIOD COVERED: October, 1985

I. Smoke Measurement by Tuneable Diode Laser

A. Water in Sidestream Smoke

The TDL system was used to measure the amount of water generated in the sidestream of three different types of cigarettes: one cigarette had an MgO treated paper, the second contained CaCO<sub>3</sub> in the paper to match the porosity of the MgO paper and the third cigarette used a standard Marlboro paper. The amount of gaseous water in the whole sidestream smoke delivered by these three cigarettes was not statistically different based on a one way analysis of variance. Plans are being considered, when time permits, to determine the total water in sidestream (i.e. vapor phase and particulate phase).

B. Formaldehyde in Mainstream Smoke

A TDL based analysis is in the process of being developed for the quantitative measurement of formaldehyde in mainstream cigarette smoke. Currently, the smoking system and transfer lines to the TDL white cell are being redesigned for this analysis.

II. Nicolet GC/FT-IR System

Performance evaluations to determine the detection limits of the "light pipe" interface as a function of molecular structure are nearing completion. The results show that the detection limits for most compounds eluting from a capillary column are on the order of 200ng per component. This is based on the ability of the search software to correctly identify the unknown in the top five best hits. Compounds which are strongly absorbing in the infrared such as low molecular weight esters may be detected in concentrations below 50ng. Weak infrared absorbers such as aliphatics may require 400 to 500ng for identification. Efforts are currently underway with Nicolet to obtain their new micro version "light-pipe" on a one for one trade basis. This new GC "light pipe" interface should enhance detection by two fold over our existing accessory.

III. Infrared Analysis of "Smokeless Cigarette"

Samples of a plastic, smokeless and non-lightable cigarette were analyzed by infrared spectroscopic techniques at the request of the Cigarette Testing Division. The results indicated that the plastic tube which resembles the rod portion of a cigarette is constructed using poly(ethylene terephthalate). The material which is contained within the plastic tube and which carries the flavor system was identified as predominately polyethylene.

**2001116501**

IV. Reports

"Gas Phase Water in Sidestream Smoke," C. N. Harward and M. E. Parrish to B. Goodman, October 28, 1985.

*R.A. Fenner*

PROJECT NUMBER: 1754  
PROJECT TITLE: Spectroscopic Studies of Tobacco and Smoke Components  
PROJECT LEADER: W. N. Einolf  
PERIOD COVERED: October, 1985

MS - (D. Magin, N. Einolf)

Cigarettes made from LTF-IIA with 10% DAP did not burn. LTF-IIA filler is now being made with 5% DAP. The LTF-IIA + 10% arbutin (hydroquinone- $\beta$ -D-glucopyranoside) cigarettes were smoked, the TPM extracted with  $\text{CH}_3\text{CN}$  and the extract reacted with BSTFA. Hydroquinone-TMS was the predominant smoke product, with a significant amount of the intact arbutin-TMS present (24% of the hydroquinone-TMS GC peak area).<sup>2</sup>

A sample of an extract from a smokeless cigarette product (FAVOR-R) was analyzed by GC/MS at the request of C. Kroustalis. Compounds identified by their mass spectrum included several hydrocarbons and related alcohols, an alkaloid with MW160, nicotine, myosmine, N-methyl-3-pyridine carboxamide, nicotine-N-oxide, nicotine-N'-oxide, nicotyrine, 4-methyl-2,6-di-tert-butyl phenol, nornicotine, cotinine, N-formyl nornicotine, and two unidentified pyridinyl ketones.<sup>3</sup>

Other samples analyzed by GC/MS included a mixture of four megastigmatrienone isomers (R. Southwick) two nopolone derivatives (D. Howe), a pyrazinyl propanone (D. Williams), two alkyl substituted oxoheptanals (D. Williams) and a dimethyl- $\alpha$ -ionone sample (F. Chappell).<sup>3</sup>

The transfer of the Wiley data base to the SS-200 data system is nearly complete, with about 61,000 spectra now entered.<sup>3</sup> Additional spectra are being entered manually by a COE student, with these spectra being added to the Flavors library.

NMR - (J. Wooten, R. Bassfield, J. Campbell)

J. Wooten made the presentation " $^{13}\text{C}$  CP/MAS NMR Spectra of Tobacco and Tobacco Constituents" at the 5th Philip Morris Science Symposium, October 18, 1985

Structural studies of carbohydrates (with G. Chan) continue with the analysis of a dibenzoyl acetylated di-mannose compound. Proton and carbon spectra are complete. Substitution of a benzoyl group in the C-2 position produces a downfield shift in H-2, thus simplifying the overlap region (3.9-4.2 ppm) of previous compounds.<sup>1</sup>

NMR analysis is complete for three of the twelve nicotine analogs involved in a chemical shift study. The measurements are being made on the N-CH<sub>3</sub>, and the 4 $\alpha$ ,  $\beta$  and 5 $\alpha$ ,  $\beta$  protons, and require the HOMCOR 2DJ experiment. This technique works well for the less severely overlapped resonances, but may not work adequately for other compounds.<sup>1</sup>

A new pulse sequence based on detecting long range proton-carbon couplings has been implemented and tested on the XL-300 NMR. This sequence may be especially useful for structural determinations of alkaloids, and is being used on model compounds.<sup>4</sup>

2001116502

REFERENCES:

1. R. Bassfield, N.B. 7398.
2. W. N. Einolf, N.B. 8040.
3. D. F. Magin, N.B. 8222.
4. J. Wooten

*W. Einolf*

PROJECT NUMBER: 1756  
PROJECT TITLE : Analytical Sensory Correlations  
PROJECT LEADER: B. W. Good  
PERIOD COVERED: October 1985

I. RL vs. RCB SMOKE

The analysis of the last three samples (two unflavored, 100% RL's from different production locations; and a 100% RL with 6.5% DAP) were completed. The data from the organic gas phase and TPM profiles were validated and transferred to the DEC 20/60 host for BMDP analysis.

II. LABSAM

A. System

Memory was increased to the maximum of 2 megabytes.

B. Online Handler

All of the RS-232 acquisition programs have been modified to store preliminary data (e.g. tares and sample weights) in keyed files. Reliability of these programs has greatly increased.

Two additional multiplexer lines were made available for the direct connection of the two balances in B-307. This reduces the demand for the six t-box lines into the system.

C. Leaf Department Reporting

Programs were written to produce memo style reports for oriental and off shore tobaccos, thus eliminating re-entry of this data into the DECSYSTEM. When Leaf assigns lot numbers, we will transfer the data into lot master tables. We will be able to send this data to IS on a mag tape, just like the bright data.



2001116503

PROJECT NUMBER: 1758  
PROJECT TITLE: Tobacco Cell Wall Research  
PROJECT LEADER: Gordon H. Bokelman  
PERIOD COVERED: October, 1985

**I. RECONSTITUTED TOBACCO STUDIES (Gordon Bokelman and George Ruben)**

A variety of reconstituted tobacco samples were platinum-carbon replicated and carbon-backed for examination by transmission electron microscopy. This study revealed that the surface of RCB consists of a coating of fibrous pectin, which is analogous to the cell wall surface of untreated tobacco. The pectin coating of RCB even covers inorganic crystals. However, RL is just the opposite; a coating of soluble components is outside, on top of the pectin layers. These findings may be relevant to the subjective characteristics of reconstituted tobaccos. That is, with different extraction conditions (such as a higher solution temperature or alkaline pH) RL might be made more similar subjectively to RCB.

**II. STRUCTURAL PROTEIN (Bill Ryan)**

The Termamyl residue, KOH residue, insoluble fraction and water-soluble fraction generated from several types of tobacco by our standard fractionation procedure were hydrolyzed with 6 N HCl and then submitted to Mike Zimmerman for HPLC amino acid analyses. This exploratory work is being undertaken as an initial investigation of the structural protein present in tobacco.

**III. PLANT SENESCENCE (Howard Sun, Pete Suiter and Robert Terrill)**

An article by Albersheim et al. [*Nature*, 314, 616-617 (1985)] reported that sycamore "oligosaccharins", molecules derived from plant cell wall biopolymers, were capable of controlling the morphogenesis of tobacco at extremely low concentrations. The results suggested that these fragments were *in situ* regulators of morphogenesis. Thus, they might have a profound influence on tobacco senescence as well.

In collaboration with Dr. Robert Terrill at the VPI & SU agricultural experimentation station in Blackstone we are interested in demonstrating that tobacco cell wall "oligosaccharins" also can control the morphogenesis of tobacco explants. To that end, the top, middle and bottom leaves of NC 22, NC 82 and Coker 319 harvested this summer were freeze-dried and Soxhlet-extracted with ethyl acetate. Then the cell wall components in the extracted residues were partially degraded by base-catalyzed hydrolysis. After neutralization and salt removal, the lyophilized extracts will be tested for their abilities to regulate morphogenesis in tobacco tissue cultures at Blackstone.

**IV. CURDLAN (Howard Sun)**

Analyses were performed on lyophilized extracellular microbial samples generated by Ernie Bravo that were thought to contain curdlan. The total carbohydrate contents of these samples, as determined by the phenol-sulfuric acid colorimetric procedure, were relatively low (10.5 to 27.9%) compared to samples submitted previously by Ernie Bravo and Debra Chadick. Aqueous

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suspensions of all but one of the samples lacked the characteristic property of curdlan to form gels upon heating to 85-90 degrees C. A sample of ATCC No. 21680 yielded a weaker gel than the standard curdlan sample (obtained from Wako Pure Chemical of Japan).

Additional investigations were performed on three of the samples. Methylation analysis revealed that these samples contained free glucose in large quantities. C-13 NMR spectra obtained by Jan Wooten and John Campbell also showed that only minor quantities of 1,3-glucans were present in these samples.

#### **V. MOLECULAR STANDARDS FOR TEM (Jeff Seeman, Gordon Bokelman and George Ruben)**

In order to determine the detection limit of the microscopy procedure employed by George Ruben, it is necessary to prepare molecular standards. We need to know the detection limit to help us interpret the electron micrographs and, hopefully, validate our tentative conclusions. We are initially planning to prepare the appropriate standards by attaching extremely long organic polymers to the surface of sub-micron silica gel beads. Ultimately we may prepare standards using sol gel beads, which have an average particle diameter of approximately 22 nm.

#### **VI. MISCELLANEOUS**

##### **A. Publications**

1. Bokelman, Gordon H., William S. Ryan, Jr., H. Howard Sun and George C. Ruben, "Tobacco Cell Wall Structural Biopolymers," Recent Advances in Tobacco Science, 11, 71-104 (1985).
2. Bokelman, Gordon H., William S. Ryan, Jr., H. Howard Sun and T. Robert Terrill, "Influence of Genetic and Cultural Factors on Chemical and Physical Properties of Tobacco: II. Cell Wall Biopolymers," accepted for publication in Beitrage zur Tabakforschung.
3. Sun, H. Howard, William S. Ryan, Jr., Jan B. Wooten, Gordon H. Bokelman and Per Aman, "Structural Characterization of a Tobacco Rhamnogalacturonan," submitted to the Philip Morris Manuscript Review Board.

##### **B. Presentations**

1. Bokelman, Gordon H., William S. Ryan, Jr., H. Howard Sun and George C. Ruben, "Tobacco Cell Wall Structural Biopolymers," presented at the Symposium of the 39th Tobacco Chemists' Research Conference in Montreal on October 3, 1985.
2. Bokelman, Gordon H., H. Howard Sun, William S. Ryan, Jr., Jan B. Wooten, Per Aman and George C. Ruben, "Structural Characterization of a Tobacco Rhamnogalacturonan," presented at the 5th Philip Morris Science Symposium on October 18, 1985.

##### **C. Memos**

1. Memo to Mr. Roland G. Leger from H. Howard Sun, "Samples for Tintometer Analysis," dated October 10, 1985.
2. Memo to Mr. H. G. Bravo from H. H. Sun, "Carbohydrate Contents of the Extracellular Microbial Samples," dated October 16, 1985.

**2001116505**

*Gordon H. Bokelman*

PROJECT NUMBER: 6505  
PROJECT TITLE: Special Investigations and Methods Development  
PROJECT LEADER: W. R. Harvey  
PERIOD COVERED: October, 1985

#### I. Metal Determination

Samples of tobacco leaves, roots and grafted tobaccos are being run for Cd determination in support of the study for Cadmium uptake.

A microwave digestion oven will be received next week and evaluated as to its merit in sample digestion.

#### II. Sugar and Alkaloid Procedure Evaluation

Assistance was given to Benson-Hedges of Canada in their establishment of a sugar and alkaloid procedure identical to ours at the Research Center. Preliminary data from B&H's determinations indicate that this effort was successful.

#### III. Cooked Flavor Support

##### A. Precook by Continuous Flow

Dispersion measurement of the flow through the above system indicates that the flow is laminar. This presents a problem with scale-up considerations.

##### B. Support for Other Cooked Flavor Endeavors

Ion chromatographic scans continue to assist these efforts.

#### IV. Volatile Acid Determination in RL Sheet

The ion chromatographic procedure for the determination of acetic and formic acids on Griffith distillates has been written and will be issued shortly.

#### V. Support for Crop Genetics International (CGI)

A standard potentiometric titration procedure for the determination of chlorides in green tobacco was adapted for CGI's needs. CGI personnel were instructed in our laboratory in its use.

#### VI. Memos and Presentations

##### A. Memos

1. J. Y. Lewis to R. H. Cox, "Potentiometric Titration of Chloride in Green Leaf Tobacco," October 10, 1985.
2. R. E. Davis and F. N. Latif, "Zymark/Hewlett-Packard Seminar," October 16, 1985.

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B. Presentations

1. Richard E. Davis, "High Performance Thin-Layer Chromatography and its Application to Alkaloids in Tobacco," Presented at the 39th Tobacco Chemists Research Conference, Montreal, Quebec, October 4, 1985.
2. Richard E. Davis, "The Determination of Nitrate in Tobacco Products Using Flow Injection Analysis," Presented at the 99th ADAC, Washington, D.C., October 28, 1985.
3. William R. Harvey and Chris S. Kroustalis, "Evaluation of a Scrubber for Phosphine for Tobacco Fumigation Chambers," Presented at the 39th Tobacco Chemists Research Conference, Montreal, Quebec, October 4, 1985.

*W. R. Harvey*

2001116507

CHARGE NUMBER: 6902  
PROGRAM TITLE: BIOCHEMICAL SPECIAL INVESTIGATIONS  
PROJECT LEADER: A. H. WARFIELD  
PERIOD COVERED: OCTOBER 1 - 31, 1985  
DATE OF REPORT: NOVEMBER 4, 1985

### I. CURING STUDIES

A. BURLEY TOBACCO (1): The burley (KY-14) tobacco will be fully cured within the next two weeks. Weekly samples have been taken from three stalk positions, and analyses are still in progress for nitrate, nitrite, nicotine, minor alkaloids, and TSNA. In addition, nitrate reductase activity (NRA) is being determined. The acquisition of analytical data should be essentially completed by the end of November. As previously reported, the objectives of this study are 1) to obtain a time-course of the fate during air-curing of nitrate and alkaloids, accumulation of TSNA, and NRA; and 2) to determine the effects of ascorbate, tocopherol, and their esters on TSNA formation during curing. After 6 weeks of curing, no NRA has been detected, although a small amount of TSNA formation has occurred, especially in the lower stalk positions. Nitrate values are generally much lower than normal for burley.

B. V-446 TOBACCO (2): Greenhouse-grown V-446 tobacco, a nornicotine converter strain, is also being air-cured on the same schedule as in the burley study. Due to the limited amount of tobacco, the sampling has been less frequent in this case. There is evidence for considerable conversion of nicotine to nornicotine during curing thus far. This study will also be completed in the next week. The objective here is to determine whether nornicotine conversion results in the formation of greatly increased levels of NNN. Although nitrate levels are low here also, NNN appears to be the major TSNA formed. The amount is very dependant on leaf position, with the highest amount occurring in the lower part of the plant. More detail will be available after the final samples are obtained. As in the burley study, no NRA has been detected.

### II. ORGANIC NITROSATING AGENTS (3)

An experiment was initiated this month to determine whether there is a possibility that organic nitrosating agents can form from constituents of the chloroform soluble portion of tobacco leaf. Burley and bright chloroform extracts have been obtained, and are being allowed to react with nitrite under various conditions. The "nitrosated" extracts will then be used to treat an amine, and the final mixtures will be analyzed for the corresponding nitrosamine. Depending on the results of the initial phase of this study, further attempts may be made to locate similar nitrosating agents formed naturally during curing.

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III. REFERENCES

1. Warfield, A. H. Notebook No. 8196; Petri, D. Notebook No. 8006; Hansen, K., Notebook No. 8215; Yu, T. Notebook No. 8149. Contributions to this study have been made by all members of 6902, as well as several members of 6908 and 1901.
2. Warfield, A. H. Notebook No. 8196; Petri, D. Notebook No. 8006; Hansen, K. Notebook No. 8215; Yu, T. Notebook No. 8149.
3. Warfield, A. H., Notebook No. 8196.

*A H Warfield*

200116509

CHARGE NUMBER: 6904  
PROJECT TITLE: BIOLOGICAL METHODS DEVELOPMENT AND UTILIZATION  
PERIOD COVERED: OCTOBER 1 - 31, 1985  
PROJECT LEADER: MIKE PENN  
DATE OF REPORT: NOVEMBER 4, 1985  
WRITTEN BY: B. D. DAVIES

#### I. V79 INHIBITION OF METABOLIC COOPERATION (IMC) ASSAY

Several experiments were conducted in an effort to reinstitute the IMC assay using a lower passage cell line. As is usual, the new cell cultures were evaluated to determine their plating efficiency and response to positive control compounds. Three assays were completed. Analyses of the results indicated that the assay was functioning normally and routine testing has begun [1].

The IMC activity of hydroquinone (HQ) was evaluated [2] using a concentration range of 0.05 to 3.0 ug/ml. As with three previous experiments involving HQ, toxicity was indicated at the higher HQ concentrations. Preliminary analysis of the data indicates that dose-related activity existed for the lower concentrations. Statistical evaluations are in progress to determine the specific activity of HQ and will be reported next month.

#### II. SOS CHROMOTEST

A previous SOS Chromotest experiment performed in-house [3] using freshly smoked CSC from six model cigarettes indicated that none of the CSCs were active, either with or without metabolic activation (S9). However, these results were questionable due to the unknown efficacy of the lyophilized (+) S9 system supplied with the kit.

In order to test whether the lack of activity was due to the lyophilized S9 or was actually a real observation, the activity of freshly-smoked 2R1 CSC was tested using both the lyophilized S9 and the frozen S9 preparation which is routinely used in-house with S/M assays [4]. Equivalent protein concentrations were used with the two S9 systems. CSC concentrations used were comparable to those used with the previous experiment. The preliminary results indicated that while there appears to be no CSC activity using the lyophilized S9, definite activity was detected when using our standard frozen S9 preparation.

Toxicity was evaluated by measuring the enzymatic activity of alkaline phosphatase (produced by the bacteria) which is not under the control of the SOS functions. The results indicated no toxicity existed when using the frozen S9, but possible toxicity existed with the lyophilized S9 at the higher CSC concentrations.

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Experiments are being conducted to confirm these preliminary observations and will be reported next month.

### III. PLANT GROWTH REGULATOR (PGR) DETERMINATIONS

An Elisa assay was conducted this month to detect the concentrations of abscisic acid (ABA) in aqueous-methanol plant extracts [5]. Comparison of the results from a standardization curve generated using a range of pure ABA concentrations with that published in the methods section of the kit indicated that the kit was working well in our hands.

To test the utility of the kit with our plant samples four samples of lamina (greenhouse senesced, greenhouse green (two different samples), and field green) were extracted using a method developed by Karen Sherwood. Several serial dilutions of the extracts were analyzed using the Elisa method. The results indicated that the concentrations of ABA in the dilutions were too high to allow an accurate determination. Plans call for preparing several more dilutions and analyzing these with the assay.

In an attempt to determine if the plant extract would interfere with the Elisa measurement of ABA, pure ABA was added to an extract dilution and analyzed using the Elisa method. The preliminary results from that experiment indicated that the plant extract does not inhibit the detection and quantitation of ABA.

### IV. REFERENCES

1. Tickle, M. H. PM Notebook 8200, pp. 136.
2. Horn, J. L. PM Notebook 8229, pp. 44-51.
3. Horn, J. L. PM Notebook 8209, pp. 123-125.
4. Horn, J. L. PM Notebook 8229, pp. 52-60.
5. Davies, B. D. Notebook 8005, pp. 189-190.

*Breed* *D. Davies*

2001116511

CHARGE NUMBER: 6906  
PROGRAM TITLE: BIOLOGICAL EFFECTS OF SMOKE  
PERIOD COVERED: OCTOBER 1 - 31, 1985  
PROJECT LEADER: D. J. AYERS  
DATE OF REPORT: NOVEMBER 1, 1985  
WRITTEN BY: W. R. MCCOY

I. SALMONELLA/MICROSOME (S/M) MUTATION ASSAY: TA98 ACTIVITY  
WITH METABOLIC ACTIVATION (+S9) OF IMPACTION TRAPPED (IT)  
CSC COLLECTED FROM A SERIES OF LOW ALKALOID FILLER CIGARETTES<sup>1</sup>

Strain TA98+S9 was used in the S/M assay to determine the specific activity (S.A.) of IT CSC obtained from a series of blended filler cigarettes. The cigarettes contained either Virginia sun cured or dark air cured tobacco blended with either normal or low alkaloid bright and burley. All of the blended filler CSC samples from the series of CSCs were lower in S.A. than the 100% dark air cured or the 100% Virginia sun cured samples. Substitution of low alkaloid material did not decrease activity. (In one case the low alkaloid significantly increased the activity).

II. S/M TA98+S9 ACTIVITY OF IT CSC COLLECTED FROM 100% BRIGHT  
CONTROL VERSUS 100% BRIGHT SUPERCRITICAL CO<sub>2</sub> EXTRACTED FILLER<sup>1</sup>

Strain TA98+S9 was used to determine the activity of IT CSC collected from 100% bright control versus 100% bright supercritical CO<sub>2</sub> extracted filler. No significant difference in CSC S.A. could be demonstrated between the 100% bright control and 100% bright supercritical CO<sub>2</sub> extracted filler samples, i.e., supercritical CO<sub>2</sub> extraction of 100% bright filler had no effect on the resulting CSC S.A. as compared to the untreated 100% bright control filler CSC.

III. S/M TA102+S9 ACTIVITY OF IT CSC COLLECTED FROM SIX MODEL CIGARETTES<sup>1</sup>

Strain TA102+S9 was used to determine the activity of IT CSC collected from the following six model cigarettes: 2R1, bright, LTF-5E, burley, RCB, and RL Ref. Four replicate samples/model were tested in order to obtain statistically significant S.A. values.

Results showed that all of the samples were active. RL Ref. and bright CSCs were lower in activity; 2R1 CSC was intermediate in activity; and burley, LTF-5E, and RCB CSCs were higher in activity. No correlation was seen between strain TA98+S9 and strain TA102+S9 results for these model CSCs. Strain TA102 appeared to have a different sensitivity to the model CSCs than did strain TA98. When strain TA102+S9 CSC activity data were qualitatively compared to GDA, L5178Y TK +/-, and IMC activity data for the model cigarettes, no correlation in the data was seen between TA102+S9 and any of these other assays. The

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reason for this observation could be due to the different assay endpoints and/or the different cell types (bacterial versus mammalian used in these assays).

#### IV. EFFECTS OF STEMS AND/OR RECONSTITUTION ON S/M TA102+S9 ACTIVITY<sup>1</sup>

Strain TA102+S9 was used to determine the effects of stems and/or reconstitution on S/M activity. The effects on strains TA98+S9 and TA100+S9 activity were previously reported.<sup>3</sup> 1T CSCs from the following model cigarettes were tested: (1) 75% DBC burley + 25% bright ES (X6D3DAM); (2) RL sheet from #1 (X6D3DFZ); (3) low solubles BW sheet from #1 (X6D3DGA); (4) 50% DBC burley + 50% bright ES (X6D3DBF); (5) RL sheet from #4 (X6D3DNY); (6) 100% bright ES (x6D3CDL); (7) RL sheet from #6 (X6D4CUC); and (8) low solubles BW sheet from #6 (X6D4ATT).

As reported for strains TA98+S9 and TA100+S9, strain TA102+S9 results showed that increased levels of bright ES in the burley-bright ES blend significantly lowered the S.A. of the CSC. In addition, no significant difference in strain TA102+S9 S.A. could be demonstrated between the CSC from any of the parent fillers and the reconstituted sheet made from those fillers.

#### V. GLUTATHIONE DEPLETION ASSAY (GDA): EFFECTS OF 2R1 CSC ON THE GLUTATHIONE (GSH) LEVELS OF V79 CELLS<sup>4</sup>

An experiment was carried out which showed that more GSH was depleted with fresh 2R1 CSC than 1, 2, or 3 hour old (stored at room temperature) 2R1 CSC.

#### VI. VALIDATION OF A METHOD TO DETERMINE TRUE TOXICITIES IN THE IMC ASSAY<sup>5</sup>

Experiments were conducted in order to validate a method for the determination of agent-induced toxicity in the IMC assay. 6-Thioguanine (6TG) was utilized to inactivate sensitive (S) cells so that the toxicity of an agent to resistant cells can be evaluated. Several 6TG pre-incubation times were tested to determine the minimum 6TG pre-incubation time necessary to prevent colony formation of 5E5 S cells. Results showed that a pre-incubation time of 44 hrs reduced S colonies to a tolerable level (12 colonies/5E5 cells), while further pre-incubation (68 hrs) reduced S colonies to the quite acceptable level of 2 colonies/5E5 cells. A test is in progress to confirm these results and to determine whether 1 or 2 rinses are needed to reduce the concentration of residual 6TG in dishes to a non-toxic level.

#### VII. REFERENCES

**2001116513**

1. McCoy, W. R. Notebook No. 8257, pp. 15-23.
2. Horn, J. L. Qualification and Standard Operating Procedures of the Salmonella/Microsome Assay Using Tester Strain TA102. Special Report, Acc. No. 85-104; 1985 May 21.
3. Ellis, C. K. 6906 Annual Report -- Biological Effects of Smoke. Acc. No. 85-095; 1985 May 17.
4. Hackett, C. J. Jr. Notebook No. 8253, pp. 43-44.
5. Garcia, H. D. Notebook No. 8202, pp. 142-168.

*W.R. McCoy*

CHARGE NUMBER: 6908  
PROGRAM TITLE: SMOKE CONDENSATE STUDIES  
PERIOD COVERED: OCTOBER 1 - 31, 1985  
PROJECT LEADER: R. D. KINSER  
DATE OF REPORT: NOVEMBER 5, 1985

## I. NITROSAMINES

Investigations of possible artifactual formation of tobacco-specific nitrosamines (TSNA) during collection of mainstream (MS) smoke were continued [1,2]. Variables which have been examined include volume of smoke passing through the traps (determined by number of cigarettes smoked), composition of purge gas (air vs. nitrogen), duration of smoking experiment, and amount of ascorbic acid (present as an inhibitor of artifactual TSNA formation) added to the traps. Results from these studies indicate that the 20 mM concentration of ascorbic acid typically utilized is not effective for smokings of 15 or more DBC burley (X6D4BVY) cigarettes. Apparent artifactual formation of NNN is noted when only five BVY cigarettes are smoked if the collection time is increased. Preliminary results suggest that large quantities of ascorbic acid (a 7.5-fold increase over usual levels) may even promote TSNA formation. Experiments are planned to determine if these effects are observed for an all bright cigarette and to develop an artifact-free trapping environment.

A nitrosatable amine, 2,6-dimethylpiperidine, was added to the smoke collection apparatus to monitor the reactions which appeared to be occurring in the bubbler traps [3]. However, no nitrosodimethylpiperidine was found by analysis of trap contents. It is possible that some other amine would be a better probe of artifactual formation; 2-ethylpiperidine has been suggested as a candidate.

A dose response curve was generated for the TSNA delivery inhibiting capability of propyl 3,4-dihydroxyhydrocinnamate (PrDHHC) [3]. Increased levels of PrDHHC resulted in lower TSNA levels in MS smoke. The maximum dosage feasible was ca. 7% w/w, and resulted in TSNA reductions of 30-40%. A similar study of tocopheryl acetate is planned. A study of the effect of alcohol chain length on the TSNA inhibiting properties of the cinnamate esters was also performed. No significant change in MS TSNA level was obtained for methyl DHHC vs. PrDHHC or PrDHHC vs. octyl DHHC. A slight difference was observed between MeDHHC and OcDHHC, but no further work along these lines is planned at this time. Application of the residue from a distillation of Spanish rosemary oil to burley tobacco resulted in decreases in TSNA levels of MS smoke; chemical characterization of the residue will be performed.

## II. CONDENSATE CHARACTERIZATION

Studies to characterize the components responsible for the undesirable subjective effects of washed, shredded bright stem have begun. Initial experiments will compare particulate depleted MS smoke with gas phase depleted MS smoke on

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a subjective and gross chemical basis. These altered vapor/particulate distribution smoke streams will be produced by using paper or charcoal filters. Cigarettes have been designed, filters located, and the filler submitted for cigarette production [4].

### III. MAINSTREAM SMOKE STUDIES

The effect of puff profile on 2R1 TPM delivery was measured this month at four different puff volumes with the Programmable Profile Smoking Machine (PPSM) [5]. After calibration of the TRIM settings, puff counts and deliveries for the seven different puff profiles were determined. Puff counts were somewhat higher at the lowest puff volume used (17.5 cc), but varied little for the other three (35, 70, 105 cc). No significant differences in deliveries were noted for any of the puff profiles for 17.5 cc and 35 cc puffs, while some variation was noted for the higher volume puffs.

Condensate from twenty-three different cigarette codes was collected in 83 smoking experiments and solutions were prepared for S/M analysis.

### IV. REFERENCES

1. Morgan, W. R. Notebook 8218, p. 116.
2. Lambert, E. A. Notebook 8240, p. 35.
3. Haut, S. A. Notebook 8167, p. 181.
4. Levins, R. J. Notebook 8248, p. 1.
5. Hellams, R. D. Notebook 8250, p. 48.

*Robin D. Kinsler*

2001116515

**2001116516**

CHARGE NUMBER: 8101  
PROGRAM TITLE: Cigarette Testing Services  
SECTION LEADER: Cynthia C. Bright  
PERIOD COVERED: October, 1985

I. ANALYTICAL SUPPORT

A. Favor, A Non-Burning, "Cigarette"-shaped Article (C. Bright, B. Kanipe, B. Joyner)

Favor is manufactured in three versions, Regular, Lights, and Menthol. The major flavor components are nicotine and menthol. In order to determine the nicotine delivery for this product, standard Cambridge filter pads were treated with 5% sodium bisulfate to enhance the affinity for nicotine in the vapor phase. The Regular and Lights versions contained approximately 8 to 9 mg of nicotine in the "flavor" impregnated plug-insert. Approximately 11 to 12 ug of nicotine were trapped on a per puff basis (standard FTC conditions) for both Regular and Lights versions. The Menthol version contained half of the total nicotine (4 to 5 mg) on the "flavor" impregnated plug-insert than the Regular and Lights versions contained. Approximately 6 ug of nicotine was trapped on a per puff basis using the treated pads. All three versions contained menthol flavors, but the Menthol version contained a significant quantity of menthol. The Menthol version contained 5 mg of menthol in the plug-insert and delivered approximately 5 ug on a per puff basis.

B. ALKALOID CONTENT OF RECONSTITUTED MATERIALS SEPARATED FROM THE CIGARETTE BLEND (C. Bright)

An evaluation of the alkaloid levels for the reconstituted materials from Philip Morris blends indicated that the alkaloid level of the recon analyzed prior to blending is lower than the alkaloid content in recon separated from the total blend. Representative blends from competitive products were also examined. The reconstituted materials separated from competitive blends all had lower alkaloid levels than the remainder of their blend, and large migration observed in P.M. blends was not seen to the same degree. The dramatic movement in the reconstituted material (0.7% alkaloids prior to blending) was shown in several Philip Morris blends (1.6% alkaloids for the reconstituted material after blending). Loew's Theaters, Inc. (Lorillard) was issued a patent in August, 1980 (#4,215,706) entitled "Nicotine Transfer Process", which documents this phenomenon especially in the presence of an ammonium salt of a strong acid (i.e. diammonium phosphate). Further work includes evaluating the degree of alkaloid migration for cigarette blends containing RCB, RL 150-B, TC, ET-Westab, and DET as blend components.

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C. RECONSTITUTED TOBACCO ANNUAL REPORT (N. Ryan)

Reconstituted tobaccos from Philip Morris and competitors' brands, manufactured during the first half of 1985, were microscopically examined for material content and type of process.

It appears that Brown & Williamson and Liggett Group are using the same type of reconstituted sheet. Their reconstituted material consists of tobacco leaf and stem as well as approximately 20-25% softwood fibers (these fibers are not alpha cellulose). Prior to January, 1984, Liggett Group used a sheet consisting of only tobacco products. Lorillard's reconstituted sheet is approximately four times thicker than any other reconstituted sheet. All companies use the papermaking process, with the exception of Philip Morris' RCB sheet, which is made by the slurry process.

II. STANDARDS LABORATORY (R. Wiley)

The canning of Monitor #24 cigarettes was completed. The total number of cans was 19,368. This smoking cigarette monitor is distributed by the Standards Laboratory to the Philip Morris testing laboratories. The calibration of this monitor has begun and will be completed by November 15, 1985.

III. VARIABLE PUFF SMOKING MACHINE (B. Mait and B. Joyner)

The variable puff smoking machine is operational. To date, ten smoking runs have been generated to establish delivery level and machine performance. The data show the machine performance is consistent in smoke delivery for the 20 ports. The machine is now being adjusted to delivery of the monitor target.

IV. MARKET ACTIVITY (B. Mait and K. Sanderson)

R. J. Reynolds is test marketing Winston Lights 80 (Box) in Birmingham, Alabama; Knoxville, Tennessee; and Fayetteville, North Carolina. This new brand is similar to Winston Lights 85 currently on the market and delivers 10 mg tar and 0.6 mg nicotine.

R. J. Reynolds is test marketing Century Lights Menthol 100 in San Francisco, California. There are nine packs in the carton for a total of 225 cigarettes per carton; thus, the consumer receives 25 free cigarettes. The tar delivery is 12 mg on this brand.

Brown & Williamson is test marketing Kool 85 in 25 cigarettes per pack in Nashville, Tennessee and Pittsburgh, Pennsylvania. This brand is similar to Kool 85 currently on the market. The price of the carton is the same as a conventional carton of cigarettes.

American Tobacco Co. is relaunching Lucky Strike Menthol 100 in test market in Birmingham, Alabama. This brand was first introduced in 1967.

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The Davidoff Cigar Co. is distributing Davidoff cigarettes in the United States. These cigarettes were manufactured by Reemstma in West Germany. These cigarettes are packaged in a black pack and will sell for a premium price in the U.S. The cigarette circumference is 27 mm and the cigarette length is 94 mm.

V. REPORTS, MEMOS, AND PRESENTATIONS

1. Memo to J. E. Wickham from H. A. Gray, "Summary of Filter Ventilation Characteristics of Domestic Brands," October 1, 1985.
2. J. F. Stargardt and G. V. Carter, "Automated Analysis of Glycyrrhizic Acid in Licorice Flavors and Cigarette Tobacco Extracts by High Performance Liquid Chromatography," presented at the 39th Tobacco Chemists' Research Conference, October 4, 1985.
3. C. C. Bright to Mr. J. E. Wickham, "Favor, a Non-Burning Cigarette Article," October 21, 1985.
4. Memo to J. E. Wickham from H. A. Gray, "Estimated Sales Weighted Averages of the Filter Ventilation Types," October 24, 1985.
5. C. C. Bright to Mr. J. E. Wickham, "Alkaloid Content of Reconstituted Materials Separated from the Cigarette Blend," October 29, 1985.

CCB:rad

C.C. Bright rep

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